

THE
AMERICAN JOURNAL
OF
PHYSIOLOGY

VOLUME 112



BALTIMORE, MD.
1935

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THE AMERICAN JOURNAL OF PHYSIOLOGY

VOL. 112

MAY 1, 1935

No. 1

PITCH AND INTENSITY DISCRIMINATION BY CATS

SIMON DWORKIN

From the Department of Physiology, McGill University, Montreal, Canada

Received for publication January 23, 1935

While much is known concerning the acoustic analyser of the dog (Pavlov, 1927), observations upon the cat have been few. Zeliony (1909) reported in one cat ability to distinguish between two notes of an organ pipe, half a tone apart. Shepherd (1914) states that cats can discriminate between notes one and two octaves apart.

In the present investigation an attempt was made to measure separately the sensitivity of the "acoustic analyser" of the cat to differences of intensity and of pitch of pure musical notes. A motor-alimentary training method was used throughout—see Dworkin (1934). During the experiments the animals were enclosed in a silence room entirely isolated from the observer. The pure musical notes were generated by means of a variable-frequency audio oscillator, and delivered by a large magnetic loud speaker placed about 1 foot from the animals. To control the loudness of the sounds, an attenuation meter calibrated in steps of 2 decibels was placed between the oscillator and loud-speaker.¹ By lighting the filament of an intermediate amplifier the sound stimuli could be turned on without accompanying clicks.

By a minimum of 200 tests the animals had all been previously trained to respond positively to a wide range of musical sounds.

The procedure used in establishing discriminations was that of "differentiating inhibition" (Pavlov). When a "positive" tone is sounded, the animal receives food, but not when a "negative" tone is sounded. Ultimately, owing to non-reinforcement, the response to the negative stimulus is abolished. Usually one, sometimes two, negative stimuli

¹ For each frequency, the output of the oscillator was set so that attenuation of 60 decibels reduced the loudness to the threshold of hearing in man. Hence a loudness of 60 decibels was produced by zero attenuation of the meter, a loudness of 10 decibels by 50 decibels' attenuation, etc.

were used per day, along with six or seven positive stimuli. The time interval between any two stimuli varied from two to five minutes.

I. *The intensity factor in pitch discrimination.* The first experiments brought out the importance of loudness in work on tone discrimination. In one cat perfect differentiation was obtained between two notes which differed not only in pitch but also in loudness. The intensity of the louder tone was then diminished, and the differentiation disappeared, although the frequency remained unaltered. On further study, it was found that whenever pitch discrimination was developed in the presence of a notable intensity difference, the significance of the notes could be reversed by reversing the intensities. For example, one cat was trained to respond positively to a frequency of 2600 cycles per second with a loudness of 60 decibels, and negatively to a frequency of 4000 cycles with a loudness of 30 decibels. Thereupon the loudness of the positive tone was reduced to about 30 decibels, and the cat received food several times while this quieter positive tone was presented. The animal remained positive. Later, when the previously negative tone, likewise of 30 decibels, was sounded, the response was now strongly positive.

Another cat was trained to respond positively to a note of 2600 cycles with a loudness of 60 decibels, and negatively to a note of 3500 cycles with a loudness of 30 decibels. Then the loudness of the 2600 cycle note was reduced to 30 decibels, whereupon the animal failed to respond. When the loudness was increased to 60 decibels, a strong positive response was again elicited.

II. *The limits of intensity discrimination by cats.* An attempt was next made to work out the limits of intensity discrimination in two cats. These animals were trained first to respond to a note of fixed frequency, and then to distinguish in that note differences of intensity.

In one cat the frequency chosen was 2600 cycles per second. For the first differentiations a loudness of 56 decibels was selected as the positive stimulus, that of 30 decibels as the negative stimulus. The distinction was made after 5 trials of the negative stimulus. At the 12th trial 48 decibels was differentiated from 30 decibels. At the 20th trial 54 decibels was distinguished from 38 decibels, and at the 23rd trial the differentiation between 54 decibels and 42 decibels was strong. From this point on the problem for the cat became more difficult. Only at the 56th trial was the animal able to distinguish 54 decibels from 48 decibels, and this distinction was at times uncertain and often absent. After 71 trials this cat was able to discriminate, at times, between 54 and 50 decibels, but the distinction was unstable. Closer differentiation, even after many further trials, was quite inconstant, so it seems reasonable to conclude that the limit of loudness discrimination in this cat was about 4 decibels.

In another cat a similar experiment was carried out, the tone-frequency

being this time 1500 cycles per second. Here the loudness of the positive signal was varied, while that of the negative was kept constant. In this cat also the limit of discrimination proved to be 4 decibels.

In both animals it was observed that, when the difference in loudness was 8 decibels or more, the differentiation was fixed or "absolute" (Pavlov), i.e., there never was any confusion on the part of the animal even when the louder of the two sounds was purposely preceded by a sound 8 decibels louder still. When the difference in loudness was less than 8 decibels, the cats occasionally mistook the negative for the positive stimulus, the confusion becoming frequent and very definite as the 4-decibel limit was exceeded. An animal that has been trained to respond positively to 54 decibels and negatively to 50 decibels, will respond negatively to 54 decibels if this signal is preceded by one of 58 or 60 decibels.

III. *Pitch discrimination in the cat.* For the work on pitch discrimination, three well trained, active cats were chosen.

A typical protocol of one of these experiments is as follows: To begin with, a note of 2000 cycles per second was chosen as the positive, a note of 3400 cycles as the negative stimulus. Complete differentiation was established at the 7th trial. The negative note was then stepped down to 3000 c.p.s. and by the 11th test this too was differentiated from 2000. Then the negative stimulus was brought down to 2900; this took 16 to 17 further trials, so that in a total of 27 to 28 negative tests differentiation between 2000 and 2900 c.p.s. was well established.

The negative note was then moved down to 2600; differentiation was complete (zero response to 2 successive negative stimuli). Now 2500 c.p.s. was made the negative stimulus. This differentiation was complete at the 50th negative test. At the 56th trial, 2360 c.p.s. was differentiated from 2000 c.p.s. Next the positive stimulus was moved down to 2150 c.p.s., so that the cat now had to distinguish 2360 c.p.s. from 2150 c.p.s. This differentiation was very difficult but was attained once at the 76th trial, was weakly evident at trial no. 77, and was really established only after the 84th test. Attempts to effect a closer differentiation were only occasionally successful.

A second cat was trained to discriminate between notes of 2500 and 2160 cycles per second in 65 tests, while a third cat was, in 77 tests, trained to distinguish a note of 2600 from 2900 cycles per second. In neither animal was it possible, even after 30 more trials, to form closer differentiations.

SUMMARY

By means of an alimentary-motor conditioning method, cats were trained to discriminate between musical notes differing in loudness and in pitch. The extreme limit of loudness differentiation was about 4 decibels, that of pitch discrimination about one tone. Whenever pitch discrimina-

tion was developed in the presence of a considerable difference in loudness, it was possible to reverse the significance of the notes by changing the intensity. Hence it is necessary when dealing only with pitch discrimination to use notes of equal loudness.

For financial assistance in this work, the author wishes to acknowledge his indebtedness to the Research Bureau of the American Otological Society.

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HEXOSEMONOPHOSPHATE CHANGES IN MUSCLE IN RELATION TO RATE OF STIMULATION AND WORK PERFORMED

ROBERT E. FISHER AND GERTY T. CORI

*From the Department of Pharmacology, Washington University School of Medicine,
Saint Louis*

Received for publication January 28, 1935

An increase in the hexosemonophosphate content of muscle after various forms of stimulation has been consistently observed in this laboratory (1), but what factors might play a rôle in the accumulation of hexosephosphate have not been investigated in detail. It was noted that when a muscle was stimulated tetanically there seemed to be a greater increase in hexosephosphate than when the stimulation was effected by single shocks, and this occurred in spite of the fact that in some cases more lactic acid accumulated during single shock than during tetanic stimulation. Since these muscles were stimulated *in situ* no measurement of work was made. However, such findings suggested that the rate of stimulation might influence the accumulation of hexosephosphate and accordingly an investigation was carried out to test this possibility.

There exist a few reports in the literature on the effect of rate of stimulation on lactic acid formation in isolated frog muscle. Lundsgaard (2) and Meyerhof and Schulz (3) found no change in the $\frac{\text{lactic acid}}{\text{work}}$ ratio with single shocks applied at different rates, while Lenhartz (4) obtained slightly more lactic acid per unit of work with slow as compared to rapid rates of stimulation. Visscher and Smith (5) reported that lactic acid produced per unit of work varied inversely with the rate of stimulation, but the element of fatigue was not ruled out in their experiments.

EXPERIMENTAL. Gastrocnemii from large grass frogs of the same shipment were used throughout each series of experiments. After spinal transection, the muscles were removed along with the femoral attachment and part of the sciatic nerve, placed in individual tubes containing 10 cc. of oxygenated Ringer's solution maintained at pH 7.2 with CO_2 - NaHCO_3 buffer, and allowed to recover after dissection for two to three hours at 20°C . One muscle was then removed from the bath, suspended in a moist chamber containing N_2 at room temperature (20 - 25°C .), and affixed to the tension lever and electrodes, after which a tension of 25 to 30 grams was

applied, the muscle length taken, and the chamber closed. After stimulation the muscle was fixed in $\text{HCl}-\text{HgCl}_2$ solution contained in tared and stoppered tubes, as described in a previous paper (6). The mate to the muscle mentioned above was taken in some instances for the determination of resting levels and in others for stimulation at a different rate.

Analyses. Hexosemonophosphate hexose and phosphorus were determined as described elsewhere (7) and lactic acid by the method of Friedemann, Cotonio, and Shaffer as modified by Wendel (8). All chemical measurements are expressed in milligrams per 100 grams muscle. The hexosephosphate hexose figures only are tabulated; the usual agreement between P calculated (from hexose figures) and P found was observed throughout. In 63 observations covering a range of 7 to 29 mgm. of hexosephosphate P per 100 grams muscle, the average P value calculated from hexose figures was 16.6 as compared to the average of 18.0 mgm. per cent from directly determined P values.

Stimulation. The rates chosen were 10 and 60 per minute, for it was soon found that these are about the limits at which one can elicit 60 contractions and avoid on the one hand contracture and fatigue, and on the other an undue difference in the length of time necessary to produce 60 twitches. The stimuli were condenser discharges timed by a neon tube circuit, and were always indirect, descending, and supramaximal.

Tension record. The tension lever consisted of a length of clock spring with a writing lever attached at right angles, and allowed the muscle to shorten about 0.7 mm. per 100 grams tension. After each experiment a direct calibration by weights suspended over a pulley was run onto the record. For single shocks tension was summated and multiplied by the length of the muscle in centimeters, and for tetani the tension-length-time product was obtained, but no correction for the arrangement of fibers in the gastrocnemius was made in either case. In order to compare tension with the chemical data (which are given per unit of muscle weight) the tension product was divided by the weight of the muscle in grams.

Total tension in the tables is expressed in kilograms per gram muscle, in which case the isometric coefficient for lactic acid ($K_{m(L)}$) is given by the expression

$$\frac{\text{total tension per gm. muscle (as tabulated)}}{\text{mgm. per cent increase in lactic acid}} \times 100.$$

Substituting values from experiment 1, table 1, $K_{m(L)} = \frac{7.6}{16.6} \times 100 = 163.10\%$. Isometric coefficients are not tabulated, but may be calculated in this way from data given in the tables.

Effect of rate of stimulation on lactic acid and hexosephosphate. In experiments 1 to 6, table 1, one of a pair of muscles was stimulated at a rate of

10 or 60 per minute, while its mate was fixed without stimulation for determination of basal values for lactic acid and hexosephosphate. In experiments 7a to 10a and 7b to 10b, table 1, the basal values could not be determined, since one of a pair of matched muscles was stimulated at a rate of 10 and the other at a rate of 60 per minute.

TABLE 1

Effect of sixty contractions against tension lever on lactic acid and hexosephosphate

Lactic acid and hexosephosphate (as hexose) are given in milligrams per 100 gram muscle. Total tension is given in kilograms per gram muscle.

EXPERIMENT NO.	RATE OF STIMULATION (PER MINUTE)	TOTAL TENSION	RESTING		STIMULATED	
			Lactic acid	Hexose-phosphate	Lactic acid	Hexose-phosphate
1	10	75	34	68	80	95
2	10	49	22	55	44	83
3	10	61	22	44	64	84
Average.....		62	26	56	63	87
Less basal.....					26	56
Increase.....					37	31
4	60	57	25	50	51	122
5	60	42	17	52	53	101
6	60	78	18	39	71	113
Average.....		59	20	47	58	112
Less basal.....					20	47
Increase.....					38	65
7a	10	46			47	67
8a	10	62			83	92
9a	10	62			61	82
10a	10	67			60	61
Average.....		59			63	75
Less basal*.....					23	51
Increase.....					40	24
7b	60	45			51	94
8b	60	49			64	110
9b	60	71			83	98
10b	60	72			70	98
Average.....		59	23	51	67	100
Less basal*.....					23	51
Increase.....					44	49

* Average basal values from experiments 1 to 6.

On comparing the effect of different rates of stimulation on lactic acid production one finds that in experiments 1 to 3, the "slow" series, with an average tension of 62 kgm. per gram muscle, 37 mgm. per cent of lactic

acid was formed, while in experiments 4 to 6, the "fast" series, with a nearly equal amount of tension, 38 mgm. per cent of lactic acid was produced. Similarly, in experiments 7 to 10, a and b, with equal amounts of tension, levels of 63 and 67 mgm. per cent of lactic acid were attained, which, after deduction of the average basal value, indicate lactic acid production of 40 and 44 mgm. per cent, respectively. It may be seen that stimulation at different rates, resulting in work of equal magnitude without fatigue, has little, if any effect on the amount of lactic acid formed as a result of contraction.

While the rate of stimulation produces no change in the amount of lactic acid formed, it has a marked effect on hexosephosphate formation. Muscles contracting at a rate of 60 per minute showed an average hexosephosphate increase which was twice that found in muscles producing a nearly equal amount of tension at the rate of 10 per minute (compare experiments 1 to 3 and 7a to 10a with experiments 4 to 6 and 7b to 10b, table 1).

In these experiments the muscles were fixed immediately after cessation of stimulation, but since 6 minutes were required for 60 contractions in the slow and only one minute in the fast series, the objection could be made that the difference in hexosephosphate observed was due to the greater length of time available for disappearance of the ester in the slow as compared to the fast series. Such a supposition could be entertained only if hexosephosphate disappeared as rapidly as 34 mgm. per cent in five minutes, but in previous experiments (1) such rates of disappearance were not found either aerobically or anaerobically. As a matter of fact, in muscle kept anaerobically there occurred a slight rise in hexosephosphate content in the first 30 minutes after stimulation. Nevertheless, it seemed advisable to test this point once more. Consequently another series of experiments was performed, identical with the "slow" and "fast" series in table 1, with the exception that the muscles stimulated at 60 per minute were allowed to remain in N_2 for 5 minutes after stimulation, so that the time elapsing between the beginning of stimulation and fixation was the same in the two series. The results (table 2) were identical in kind with those in table 1. For similar amounts of tension (64 and 68 kgm. per gm. muscle) similar levels of lactic acid (74 and 73 mgm. per cent) were attained; yet the rapidly stimulated muscles contained on an average 31 mgm. per cent more hexosephosphate than did those stimulated slowly.

Comparison of tetani and single contractions. It seemed of interest to determine whether tetanic stimulation would have a still greater effect on hexosephosphate increase than single shock stimulation at the rate of 60 per minute. Since the two forms of contraction cannot be compared on the basis of tension (because the tension-time product is determined in tetani and not in twitches), it was necessary to make the comparison on the basis of some chemical change related to tension such as phospho-

creatine breakdown or lactic acid formation. Tetani of 10 seconds' duration were chosen because such tetani produced about the same amount of lactic acid as did 60 twitches.

In 18 such experiments the average amount of lactic acid formed was 39, as compared to 46 and 43 mgm. per cent in 60 single contractions at 60 and 10 per minute, respectively (see table 3). Per milligram lactic acid formed

TABLE 2

Influence of rate of stimulation on lactic acid and hexosephosphate in muscles with resting periods of equal length

A. Stimulated 60 times at 10 per minute and fixed immediately.

B. Stimulated 60 times at 60 per minute and fixed after 5 minutes in nitrogen.

Lactic acid and hexosephosphate (as hexose) are given in milligrams per 100 gram muscle. Total tension is given in kilograms per gram muscle.

EXPERIMENT NO.	TOTAL TENSION		LACTIC ACID		HEXOSEPHOSPHATE	
	A	B	A	B	A	B
11	83	86	81	87	69	112
12	53	64	86	79	77	104
13	55	54	55	53	54	79
Average..	64	68	74	73	67	98

TABLE 3

Relation of lactic acid and hexosephosphate changes during contraction

TYPE OF STIMULATION	10 SECONDS TETANUS	60 SINGLE SHOCKS	
		60 per minute	10 per minute
Number of experiments.....	18	15	10
Lactic acid formed, mgm. per cent.....	39	46	43
Hexosephosphate formed, mgm. per cent hexose.....	63	54	25
Sum of lactic acid and hexosephosphate.....	102	100	68
Mgm. hexosephosphate per mgm. lactic acid.....	1.6	1.2	0.6
Mgm. carbohydrate* broken down per mgm. lactic acid formed.....	2.6	2.2	1.6

* Sum of lactic acid and hexosephosphate formed.

the increase in hexosephosphate was greater in the tetani than in the single contractions at 60 per minute, showing that the full effect of rate had not been reached with the latter form of stimulation.

Since hexosephosphate is formed from glycogen and accounts, together with lactic acid, for most of the glycogen disappearing as the result of contraction (10, 11), it seemed permissible to interpret the above results in terms of glycogen. In table 3, the total carbohydrate (or glycogen) broken down per unit of lactic acid formed has been calculated. The figures

show that the largest amount of glycogen is broken down in tetani, less in single contractions at 60 per minute, and still less in 60 contractions at 10 per minute.¹

Relation of length of tetanus and degree of isometrism to after-formation of lactic acid. The comparison of tetani and single contractions on the basis of lactic acid production, as was done in table 3, would not be correct if the conditions of the experiments permitted an appreciable amount of the lactic acid formed to appear after the contraction. Delayed formation of lactic acid in the case of muscles stimulated at 60 per minute has already been ruled out by the experiments in table 2, where a resting period of 5

TABLE 4

Relation of delayed formation of lactic acid to length of tetanus and degree of isometrism

Matched muscles, (1) fixed 4 to 8 seconds after stimulation; (2) fixed 3 minutes after stimulation. Lactic acid and hexosephosphate (as hexose) are given in milligrams per 100 gram muscle. Total tension is given in kilogram-seconds per gram muscle. All figures are averages.

	GROUP					
	A		B		C	
Number of experiments.....	5		3		4	
Length of tetanus (sec.).....	3-6		10		10	
Shortening per 100 gm. tension (mm.)....	0.7		0.7		0.04	
Muscle.....	1	2	1	2	1	2
Total tension.....	10	10	21	21	44	39
Lactic acid.....	35	41	62	66	65	71
Hexosephosphate.....	76*	80*	126**	120**	136**	145**
Lactic acid increase†.....	12	18	39	43	42	48
Lactic acid formed per unit of tension....	1.2	1.8	1.86	2.05	0.95	1.23
After formation in per cent of total.....		33		9		23

* Four experiments averaged.

** Single experiment.

† See table 1 for basal values.

minutes after stimulation did not result in additional formation of lactic acid. In 3 experiments (group B, table 4) matched muscles were tetanized for 10 seconds against the tension lever used in the previous experiments, one muscle being fixed within 6 seconds while the other was allowed to remain in N₂ for 3 minutes after stimulation. The average $\frac{\text{lactic acid}}{\text{tension}}$ ratios in these experiments indicate a delayed formation of lactic acid amounting to but 9 per cent of the total.

¹ These differences need not necessarily be reflected in heat measurements because the heat liberated in the reactions glycogen \rightarrow hexosephosphate is small as compared to the reactions glycogen \rightarrow lactic acid.

In view of the slight after-formation observed under these conditions, an attempt was made to define conditions under which it occurred. Muscles were subjected to short (3 to 6 second) tetani against the same spring, and in this case a delayed formation of 33 per cent of the total (group A) was observed. This suggested that with similar initial tensions the after-formation of lactic acid becomes greater as the time allowed for completion of the chemical changes leading to lactic acid becomes shorter. In such a case it should also be possible to increase after-formation of lactic acid in a long tetanus by allowing the muscle to produce more tension. A more nearly isometric lever and optical registration was used in the experiments in group C, allowing the muscle to shorten 0.04 mm. per 100 gram tension as compared to 0.7 mm. per 100 gram tension for the lever used in groups A and B. A definite delayed lactic acid formation was found amounting to 23 per cent of the total amount of lactic acid formed. These experiments support another observation which relates delayed lactic acid formation to tension.²

Relation of lactic acid and hexosephosphate to work. It was noted that, in general, the increase in lactic acid seemed to be roughly proportional to the tension, while hexosephosphate was not so related. Because the nature of the experiments prevented the determination of basal values for each experiment, averages only could be used to demonstrate this observation. The experiments were tabulated in an ascending order of work, and averages for tension, lactic acid, and hexosephosphate were calculated for the first and second half of each group of experiments. From the average levels the basal values (table 1) were deducted to find increases in lactic acid and hexosephosphate. Columns 2 and 3 in table 5 represent the average figures so obtained.

In column 1 are presented data from experiments designed to produce contraction with the least possible amount of work. The muscles were stimulated without being attached to the tension lever so that their work consisted mainly in overcoming viscosity and gravity. While only a small amount of lactic acid (8 and 9 mgm. per cent) was formed, there was a considerable increase in hexosephosphate in both single shock and tetanic experiments (31 and 24 mgm. per cent, respectively). The much greater increase in hexosephosphate than in lactic acid indicates that hexosephosphate formation may be a process not entirely associated with work performed by a muscle. This suggestion is supported by experiments in which epinephrine was allowed to act on isolated frog muscle (6). Under

² Meyerhof and Schulz failed to observe delayed lactic acid formation in one series of experiments (9), while subsequently (3) they succeeded in demonstrating this phenomenon. These authors ascribed the failure to observe delayed lactic acid formation to the fact that much lower tensions were developed in the former than in the latter series of experiments.

anaerobic conditions epinephrine caused the formation of about 5 mgm. of hexosephosphate per milligram of lactic acid, a ratio similar to that produced by contractions without load. In the latter case some work is undoubtedly performed, and some phosphocreatine might be expected to be broken down, while in the former case epinephrine did not cause a decrease in phosphocreatine.

TABLE 5

Relation of lactic acid and hexosephosphate to work

Column 1, from experiments without load. Column 2, first half, and Column 3, second half of series of tension lever experiments. See text. Lactic acid and hexosephosphate (as hexose) are given in milligrams per 100 gram muscle. All figures are averages.

	60 CONTRACTIONS IN 1 MINUTE		
Column number.....	1	2	3
Number of experiments averaged.....	3	8	8
Total tension (kgm. per gm. muscle).....		54	76
Lactic acid increase.....	8	39	54
Hexosephosphate increase.....	31	52	55
Lactic acid formed per unit of tension.....		0.72	0.71
Mgm. hexosephosphate formed per mgm. increase in lactic acid.....	3.9	1.3	1.0
Mgm. carbohydrate* broken down per mgm. lactic acid formed.....	4.9	2.3	2.0
	10 SECOND TETANI		
Column number.....	1	2	3
Number of experiments averaged.....	3	9	9
Total tension (kgm.-sec. per gm. muscle).....		20	31
Lactic acid increase.....	9	33	46
Hexosephosphate increase.....	24	59	66
Lactic acid formed per unit of tension.....		1.6	1.5
Mgm. hexosephosphate formed per mgm. increase in lactic acid.....	2.7	1.8	1.4
Mgm. carbohydrate* broken down per mgm. lactic acid formed.....	3.7	2.8	2.4

* Sum of lactic acid and hexosephosphate formed.

The ratios $\frac{\text{lactic acid}}{\text{tension}}$ in columns 2 and 3 in table 5 indicate that lactic acid formed was proportional to tension within the range of these experiments. Such a proportionality does not exist in the case of hexosephosphate. Since no measurements of tension could be made in the case of contractions without load the $\frac{\text{hexosephosphate}}{\text{lactic acid}}$ ratio has been calculated. This ratio shows that in both single contractions and tetani, the greatest

increase in hexosephosphate per milligram of lactic acid occurred when the work done was very slight (contractions without load); that with intermediate amounts of tension less hexosephosphate was formed per milligram of lactic acid; and that with large amounts of tension still less was formed. In terms of glycogen this means that the amount broken down per milligram of lactic acid (or per unit of tension) decreases as the work performed increases.

The expenditure of glycogen during contraction depends not only on the work performed by a muscle but also on the rate of stimulation. Both these factors must be taken into account in order to determine the conditions under which a muscle can perform the greatest amount of work with the least expenditure of glycogen. The optimal conditions have not as yet been determined, but the experiments show that single contractions at a slow rate, with each contraction producing a great deal of tension, cause the smallest breakdown of glycogen per unit of work. More glycogen per unit of work is expended in the same number of contractions at a more rapid rate producing the same amount of work, or contractions at the slow rate producing less work. Still more glycogen is broken down in tetani, while relatively the greatest expenditure of glycogen occurs in contractions without load.

SUMMARY

1. Sixty single contractions of isolated frog gastrocnemii against a tension lever led to the production of equal amounts of work and lactic acid when the rate of stimulation was either 10 or 60 per minute. In the former case, however, the increase in hexosemonophosphate was about half that in the latter case. A single ten second tetanus produced approximately the same amount of lactic acid, and still more hexosephosphate than did the contractions at 60 per minute. Since hexosephosphate is formed from glycogen, it follows that a greater breakdown of glycogen occurs in rapidly than in slowly stimulated muscles performing equal amounts of work.

2. In 60 single contractions or a ten second tetanus of an unloaded muscle about half as much hexosephosphate and one-fifth as much lactic acid was formed as in similar contractions of a muscle against a tension spring.

3. In contrast to lactic acid, hexosephosphate was not directly related to the work performed. Per milligram of lactic acid formed the increase in hexosephosphate was greatest in contractions without load and became smaller for increasing amounts of work. It may be concluded that, considering the effects of both rate of stimulation and work, a muscle can produce the greatest amount of work with the least expenditure of glycogen if it is producing maximal tension at a slow rate of stimulation.

4. Significant delayed formation of lactic acid was not observed after 10

second tetani against a spring permitting considerable shortening of the muscle. After-formation was observed, however, following short tetani against a weak spring, and following 10 second tetani against a stiff tension lever. In no case was there a significant delayed formation of hexose-phosphate.

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THE ORIGIN AND SIGNIFICANCE OF NEUTRAL CHLORIDE IN THE SECRETIONS OF THE STOMACH AND DUODENUM

CHARLES M. WILHELMJ, LEO C. HENRICH, IRWIN NEIGUS AND
FREDERICK C. HILL

*From the Departments of Physiology and Experimental Surgery, Creighton University
School of Medicine, Omaha, Nebraska*

Received for publication January 24, 1935

During the past three years a large number of experiments have been performed in this laboratory in which standard hydrochloric acid solutions have either been introduced into pouches of various parts of the stomach and duodenum or have been mixed with the secretions from these parts. An analysis of these data has thrown considerable light on the origin and significance of the neutral chloride present in gastric contents. The present communication presents an analysis of these experiments.

MATERIAL. The present analysis is based on 95 experiments comprising 12 on whole stomach pouches; 20 on isolated pyloric pouches; 19 on isolated duodenal pouches; 21 on the secretions from the intact duodenum obtained after stimulation with acid; 12 in dogs with gastroduodenostomy; 11 in dogs with gastrojejunostomy just below the ligament of Trietz.

METHODS AND CALCULATIONS. The methods and calculations have been described in detail in previous publications and will only be briefly outlined here. The general procedure was to introduce a standard hydrochloric acid solution (approximately tenth normal) containing phenol red, into the pouch to be studied and to allow it to remain for one half-hour; at the end of one half-hour the pouch was emptied as completely as possible and fresh acid solution introduced, at the end of the second half-hour period the pouch was again refilled. From three to four half-hour samples were usually obtained consecutively. In the whole stomach pouches and in the stomachs with gastroduodenostomy and gastrojejunostomy, the stomach was not stimulated with histamine and only those experiments in which there was no evidence of secretion of hydrochloric acid by the stomach have been considered in the present analysis. In the experiments with duodenal secretions obtained from the intact duodenum, the procedure was to stimulate the duodenum by introducing the hydrochloric acid-phenol red solution through a duodenal tube; when a copious flow of secretion occurred the secretions were removed and mixed with the same

TABLE 1

ACID SOLUTION USED, MGM. ACID CHLORIDE PER 100 CC.	P.S.P., PER CENT	TOTAL NEUTRAL CHLORIDE, MGM. PER 100 CC.	NEUTRAL CHLORIDE FROM NEU- TRALIZED ACID, MGM. PER 100 CC.	EXTRA NEUTRAL CHLORIDE, MGM. PER 100 CC.	PER CENT NEUTRAL CHLORIDE FROM NEU- TRALIZED ACID	CHLORIDE CON- CENTRATION OF SECRETION, MGM. PER 100 CC.	EXPERIMENT
349	98	15	6	9	40	450	Whole stom- ach pouches
	98	15	9	6	60	300	
351	93	30	5	25	17	357	
	96	26	9	17	35	425	
	96	24	13	11	54	275	
351	96	17	3	14	18	350	
	96	19	7	12	37	300	
351	93	28	7	21	25	300	
	89	46	11	35	24	319	
351	94	21	6	15	29	250	
	89	39	3	36	8	328	
	88	66	23	43	35	358	
Average.....					32	334	
348	79	123	47	76	38	362	Pyloric pouches
	84	81	13	68	16	425	
	85	79	13	66	16	440	
	83	92	29	63	32	370	
	81	99	24	75	24	395	
	81	98	28	70	29	370	
	78	139	40	99	29	450	
	75	134	30	104	22	416	
	71	141	23	118	16	407	
	84	61	9	52	15	325	
	92	41	13	28	32	350	
	91	67	40	27	60	300	
	86	60	4	56	7	400	
	89	48	13	35	27	318	
	61	211	71	140	34	359	
	70	143	38	105	27	350	
	70	143	34	109	24	363	
	67	162	43	119	27	361	
	74	121	28	93	23	358	
	80	126	58	68	46	340	
Average.....					27	373	
Average of 32 experiments.....					29	358	

TABLE 2

ACID SOLUTION USED, MGM. ACID CHLORIDE PER 100 CC.	P.S.P., PER CENT	TOTAL NEUTRAL CHLORIDE, MGM. PER 100 CC.	NEUTRAL CHLORIDE FROM NEUTRALIZED ACID, MGM. PER 100 CC.	EXTRA NEUTRAL CHLORIDE, MGM. PER 100 CC.	PER CENT NEUTRAL CHLORIDE FROM NEUTRALIZED ACID	CHLORIDE CONCENTRATION OF SECRETION, MGM. PER 100 CC.	EXPERIMENT
348	55	172	45	127	26	282	Isolated duodenal pouches
	65	152	61	91	40	260	
	65	162	82	80	51	230	
	62	187	84	103	45	271	
	69	170	87	83	51	268	
	63	188	94	94	50	254	
	48	206	37	169	18	325	
	61	186	63	123	34	315	
	58	191	63	128	33	305	
	54	166	4	162	2	352	
	58	177	39	138	22	329	
	65	165	43	122	26	349	
	60	165	33	132	20	330	
	57	188	52	136	28	316	
	50	214	32	182	15	364	
	53	211	47	164	22	345	
	56	229	75	154	33	327	
	58	231	91	140	39	333	
	46	302	120	182	40	336	
Average.....					31	310	
350	81	103	39	64	38	336	Duodenal secretions from intact duodenum
	79	89	8	81	9	386	
	82	93	25	68	27	377	
	85	75	22	53	29	353	
348	63	201	90	111	45	300	
	61	188	76	112	40	288	
	63	195	86	109	44	295	
347	82	96	38	58	40	322	
	82	100	53	47	53	261	
	88	47	17	30	36	250	
	82	84	26	58	31	322	
347	70	125	38	87	30	290	
	71	125	23	102	18	352	
	77	113	41	72	36	313	

TABLE 2—*Concluded*

ACID SOLUTION USED, MGM. ACID CHLORIDE PER 100 CC.	P.S.P., PER CENT	TOTAL NEUTRAL CHLORIDE, MGM. PER 100 CC.	NEUTRAL CHLORIDE, FROM NEUTRALIZED ACID, MGM. PER 100 CC.	EXTRA NEUTRAL CHLORIDE, MGM. PER 100 CC.	PER CENT NEUTRAL CHLORIDE FROM NEUTRALIZED ACID	CHLORIDE CONCENTRATION OF SECRETION, MGM. PER 100 CC.	EXPERIMENT
352	82	54	4	50	7	278	Duodenal secretions from intact duodenum— <i>Concluded</i>
	86	50	11	39	22	278	
	89	44	21	23	48	210	
	86	46	13	33	28	236	
348	76	95	17	78	18	325	
	79	89	19	70	21	334	
	79	80	9	71	11	338	
Average.....					30	307	
Average of 40 experiments.....					30	308	

strength acid-phenol red solution that had been introduced into the duodenum.

The following analyses were made on the acid-phenol red solution containing the secretions: 1. The per cent of phenol red present after removal of interfering substances with 10 per cent sodium tungstate and two-thirds normal sulfuric acid. 2. Total and neutral chloride after careful ashing of the dried aliquot sample.

The calculations were as follows: The decrease in the per cent of phenol red in the mixture of acid and secretion showed how much secretion was present in 100 cc. of the mixture. When the acid chloride concentration of the original acid solution was multiplied by the per cent of phenol red in the sample, the result gave the original acid solution corrected for dilution. The total chloride concentration of the sample removed from the pouch was always above that of the original acid solution corrected for dilution, and the difference represented the chloride content of the secretions which were mixed with the acid-phenol red solution. When the extra chloride was divided by the amount of secretion in the sample (obtained from the decrease in the per cent of phenol red) the result gave the chloride concentration of the secretions. The chloride present in the secretions was neutral chloride. The acid chloride concentration of the sample removed from the pouch was always below that of the original acid solution corrected for dilution, and the difference represented hydrochloric acid that had been neutralized by the secretion of the pouch; the chloride of this neutralized acid was, of course, present with the neutral chloride fraction.

The total neutral chloride in the sample removed from the pouch could thus be divided into two fractions: a, the neutral chloride which was a constituent of the secretion, and b, neutral chloride which resulted from neutralization of hydrochloric acid by the secretions. From these data

it was possible to calculate the per cent of the total neutral chloride which resulted from neutralization of hydrochloric acid by the secretions of the pouch.

TABLE 3

ACID SOLUTION USED, MGM. ACID CHLORIDE PER 100 CC.	P.S.P., PER CENT	TOTAL NEUTRAL CHLORIDE, MGM. PER 100 CC.	NEUTRAL CHLORIDE FROM NEUTRALIZED ACID, MGM. PER 100 CC.	EXTRA NEUTRAL CHLORIDE, MGM. PER 100 CC.	PER CENT NEUTRAL CHLORIDE FROM NEUTRALIZED ACID	CHLORIDE CONCENTRATION OF SECRETION, MGM. PER 100 CC.	EXPERIMENT
348	75 78 79	134 119 116	55 51 46	79 68 70	41 43 40	316 309 333	Gastroduodenostomy
351	78 76 77	94 138 122	17 57 44	77 81 78	18 41 36	350 337 339	
348	68 67 69	111 118 103	12 16 9	99 102 104	11 14 9	310 310 303	
351	65 69 73	127 116 111	25 21 34	102 95 77	20 18 31	291 307 286	
Average.....					27	316	
349	74 77 81	114 109 100	30 42 40	84 67 60	26 39 40	323 292 316	Gastrojejunostomy
349	78 77 82	87 111 102	17 40 46	70 71 56	20 36 45	318 309 311	
351	71 81	147 89	49 29	98 60	33 33	338 316	
351	61 72 81	142 98 80	5 8 12	137 90 68	4 8 15	351 322 358	
Average.....					27	323	
Average of 23 experiments.....					27	319	

RESULTS. The results are given in tables 1, 2, and 3. In column 7 of each table is shown the chloride concentration of the secretion under consideration. It is seen that the non-acid secretions of the whole stomach and the secretions of the pylorus (table 1), the mixed duodenal secretions (bile, succus entericus and pancreatic juice) (table 2), and the combined

duodenal and non-acid gastric secretions (table 3) have approximately the same chloride concentration, the average values for each secretion ranging from 373 to 307 mgm. of neutral chloride per 100 cc. The slightly higher value for the chloride concentration of the non-acid secretion of the stomach has again been confirmed in recent unpublished experiments. The average value for the 95 experiments shown in tables 1, 2 and 3 is 327 mgm. per 100 cc.

The per cent of the total neutral chloride which is the result of neutralization of hydrochloric acid by the various secretions, is shown in column 6 of each table. The average values for the individual groups of experiments range from 27 to 32 per cent, and show relatively little differences between the secretions of the various parts of the stomach and of the duodenum. The average value for the 95 experiments is 29 per cent.

DISCUSSION. Since all of the secretions investigated may at times be found in the stomach, it is quite obvious that it is a definite error to consider that all of the neutral chloride present in the gastric contents represents neutralized hydrochloric acid; on the average, approximately only one-third of the neutral chloride actually represents neutralized hydrochloric acid, the remaining two-thirds having entered the stomach as a constituent of the various non-acid secretions.

The chloride concentration of the non-acid secretions of the stomach and the mixed duodenal secretions is very close to the blood chloride level. Previous studies (1, 2, 3, 4), have shown that the alkalinity of these various secretions is approximately the same, the average value being 0.04 normal.

The values for the neutral chloride concentration and for the alkalinity of the various non-acid secretions which may at times enter the stomach are so similar that in any given case it would be practically impossible to tell whether they were of intragastric or duodenal origin.

SUMMARY

In a series of 95 experiments it was found that the neutral chloride concentrations of the non-acid secretions of the stomach and the mixed duodenal secretions were approximately the same, the average values ranging from 358 to 307 mgm. per 100 cc.

When the non-acid secretions of the stomach or the duodenal secretions are mixed with tenth normal hydrochloric acid, only about one-third of the neutral chloride fraction represents neutralized hydrochloric acid.

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STUDIES ON THE RATE OF DISAPPEARANCE AND FATE OF MARE GONADOTROPIC HORMONE FOLLOWING INTRAVENOUS INJECTION

H. R. CATCHPOLE, H. H. COLE AND P. B. PEARSON

From the College of Agriculture, University of California, Davis, California

Received for publication January 23, 1935

Gonad-stimulating hormones are known to circulate in quantity during pregnancy in the blood streams of two widely separated mammalian groups: namely, man and the higher apes, and the family Equidae (horse, ass and zebra). The hormone of the first group has been investigated widely as prolان; that of the second group has been dealt with, as regards the conditions of its appearance, its distribution in the mare, and its properties, in a series of papers cited by Catchpole and Lyons (1934). Prolان and the mare gonadotropic hormone show marked physiological differences which have been enumerated in the above reference. The present paper concerns a further property of the mare serum hormone in which its difference from prolان is emphasized. It is well known that prolان passes the kidney with ease; its concentration in the urine of pregnant women agrees with its concentration in the blood stream itself. Parkes and White (1933) injected prolان into rabbits intravenously and studied in a roughly quantitative manner the passage of hormone into the urine. They reported that one-third or less of the injected hormone was excreted in 9 hours, and that the blood renal threshold for prolان, if any, was low. The fate of the remaining two-thirds was undetermined in their experiments. There might have been some destruction in the blood, and also the excretion might have continued after 9 hours. Their conclusion that the failure of the pregnant rabbit to excrete prolان was due to a lack of hormone in the blood was justified only as far as hormones of the prolان type are concerned. Hormones of the pregnant mare type do not pass through the kidney. The first indication of this was a practical failure to recover gonadotropic hormone from the urines of pregnant mares, even at periods when the concentration of hormone in the bloods of the animals was very high. Rarely, small amounts of hormone do appear in the urine. The figures of Zondek (1931) probably refer to one of these exceptional occasions and are not of general significance. A number of qualitative experiments by various authors bear out this difference between prolان and the mare gonadotropic hormone. Evans (1933) found that prolان was recover-

able from urines of rats injected with this substance, and Ehrhardt (1930) found that a non-pregnant woman excreted prolactin transfused from a pregnant one. Evans interestingly observed that a rhesus monkey excreted hormone after the injection of a preparation from hypophysis itself (of the pig). But both rats and monkeys failed to excrete active urines following injection of mare gonadotropic hormone. The relative difficulty of excretion of mare hormone by the kidney raises the question of the fate of the hormone in the animal body. This paper is concerned with the rate of disappearance and the fate of pregnant mare hormone following intravenous injection of rabbits and gelding.

EXPERIMENTAL. Massive doses, as expressed in rat units, of potent pregnant mare blood sera were injected into the blood streams of the experimental animals. After a short interval of time, to allow thorough mixing throughout the circulatory system, an initial blood sample was withdrawn, and thereafter samples withdrawn at fixed intervals of time. These samples were assayed for their gonadotropic hormonal content by a method similar to that proposed by Cole et al. (1932). The rat unitage was found by giving a series of dilutions of the order of 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$ cc., etc. In the gelding, where plenty of serum was available, a rather more accurate estimate was made by further grading the dose in the vicinity of the rat unit level. After the first few assays, the approximate unit could be roughly predicted, and many of the initial assays eliminated.

Disappearance and fate of mare gonadotropic hormone in the rabbit. Young New Zealand white female intact and castrated rabbits were used. It was soon found that ovariectomy produced no difference in the picture of hormone disappearance showing that the gonads do not contribute appreciably to the rapid destruction of hormone which occurs. The intact animals responded by ovulation to the injection of hormone, but the amount of hormone, if any, used up to effect this must be an insignificant portion of the total amount injected. We know of no other data on actual utilization of gonadotropic hormones by the gonads.

Most of the animals were injected in a left ear vein with 30 cc. of pregnant mare serum containing 100 rat units per cc., a total of 3,000 rat units. Usually, the only untoward effect on the animal was a transient increase in the respiration, but in two cases we encountered immediate death from shock. Fifteen to 30 minutes after the injection a 1 cc. sample of blood was withdrawn from the other ear. This amount was sufficient since the initial concentration reached by the hormone in the serum of the rabbit was as high as 40 rat units per cc.¹ The size of the samples taken was kept to a minimum to avoid the removal of a significant amount of hormone by this route. Later, when the concentration of hormone had fallen considerably, it was necessary to obtain larger samples. Sampling was done at 24 hour intervals, and was continued till the 120th or 150th hour, when

¹ This figure agrees with estimates based on the blood volume of these rabbits.

the amounts of hormone became too small to estimate. In some preliminary experiments only 1,000 rat units were injected, but the smaller concentrations of hormone made estimation less accurate. The curves relating hormone disappearance to time, and the logarithm of hormonal concentration to time, for one group appear in figure 1. In two cases the experiments were conducted in metabolism cages. The urine collected up to 144 hours after injection was precipitated in acetone and the resultant

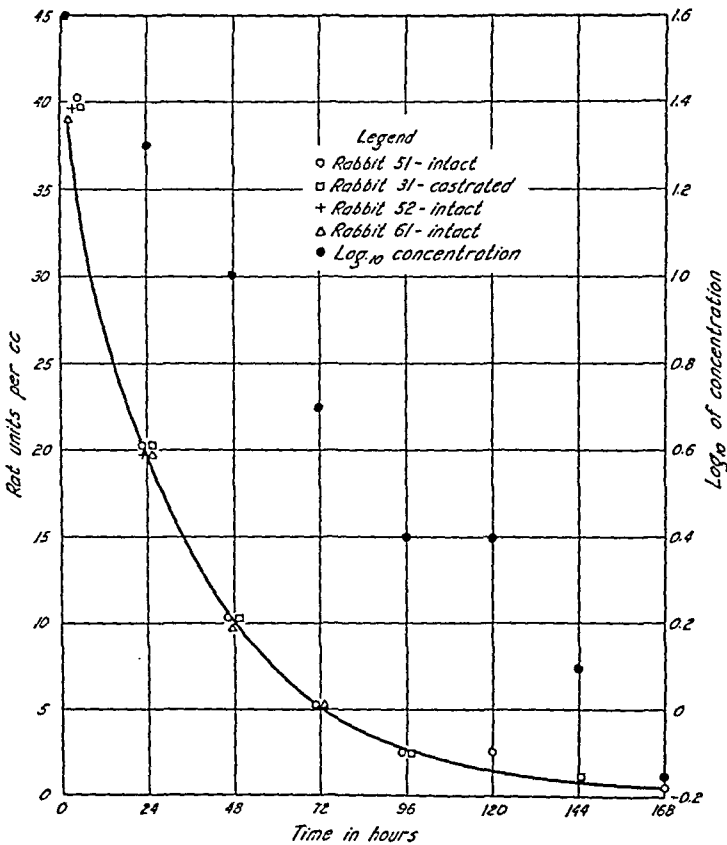


Fig. 1. Disappearance of mare gonadotropic hormone from blood of rabbits following intravenous injection. Each of the four rabbits received an intravenous injection of 3,000 R.U.

powder extracted for gonad-stimulating hormone. Doses equivalent to $\frac{1}{10}$ of the total urine given to immature rats elicited no response, although over the time that the urine was collected practically 3,000 rat units had disappeared from the body. Nor was hormone detected in feces, but all preparations from this source were almost too toxic to assay.

Two animals were sacrificed at 24 hours, following the usual injection of 3,000 rat units. At this time, approximately 1,500 rat units remained in the blood serum at a concentration of 20 rat units per cc. and 1,500 rat

units had disappeared. The uterus, spleen, lungs, kidneys, and liver of each of these animals were removed and each tissue separately extracted for gonad-stimulating hormone by the method of Catchpole and Lyons (1934). Injection of 1 gram equivalents of these glands and tissues in no case evoked a response in the test animals, proving that there was no appreciable storage of hormone at any of these sites.

Disappearance of mare gonadotropic hormone from the gelding. It was decided to continue the line of experimentation followed above by studying the course of pregnant mare serum hormone destruction in the horse itself, following its introduction into the blood stream. The proteins introduced in this case were more nearly homologous with the blood proteins of the recipient than in the rabbit experiments. Further we had a series of figures upon the rate of disappearance in the pregnant mare with which the results might profitably be compared. In the pregnant mare, after maintaining a plateau of high hormonal concentration, the hormone level starts to drop at about the 100th day of gestation (Cole and Saunders, 1935). Should the hormonal production in the mare suddenly cease, then the condition in the mare will be the same as if a certain amount of hormone had been introduced into the blood and left to disappear, and the rate of this disappearance should parallel that of an injected animal.

We used a castrated horse (gelding) weighing 1,100 pounds. About 3 liters of mare serum, containing about 90,000 rat units of hormone, were introduced into the left jugular vein with a large needle. The animal exhibited rather alarming systemic effects for some time after the injection, with marked increase in the respiration, and intestinal movements with defecation, reminiscent of anaphylactic shock. There was an edema of the breast and stiffness in the joints for about two weeks. After the first day the appetite was normal. A blood sample was withdrawn from the right jugular vein after the lapse of 1 hour. Samples were taken at 24 hour intervals for 1 week, then at 2 or 3 day intervals, and finally at 6 day intervals. Sampling was continued for 27 days in all, by which time the level of hormone had fallen so low that without extractive measures, an inconveniently large amount of serum had to be injected into rats to elicit a response.

The initial concentration of hormone in the blood serum produced by the injection of some 90,000 rat units into a horse of this size was 5 rat units per cc., a figure sufficiently close to that expected by calculation from the blood volume of the horse. It may be compared with the figure of 50 to 100 rat units per cc. reached in the pregnant mare. Figure 2 shows the curve of hormonal concentration plotted against time for the injected gelding.

DISCUSSION. In spite of the lack of appreciable urinary excretion, mare gonadotropic hormone introduced into the circulation disappears from it relatively rapidly, both in the intact and castrated animal. The curves

showing the relation between concentration and time are by no means simple in form. Cole and Saunders (1935) showed that hormone disappearance from the pregnant mare followed a logarithmic curve, with the concentration being halved every 8 to 10 days. In the gelding, there is a fall in hormonal concentration to $\frac{1}{2}$ in the first 72 hours after injection, but thereafter the rate of destruction decreases and during the next 20 days there is a rather regular fall in hormone to $\frac{1}{2}$ every 6 days. There is thus a definitely greater destruction rate in the gelding than in the mare, a difference which may be a real one, or more probably due to a persistence of

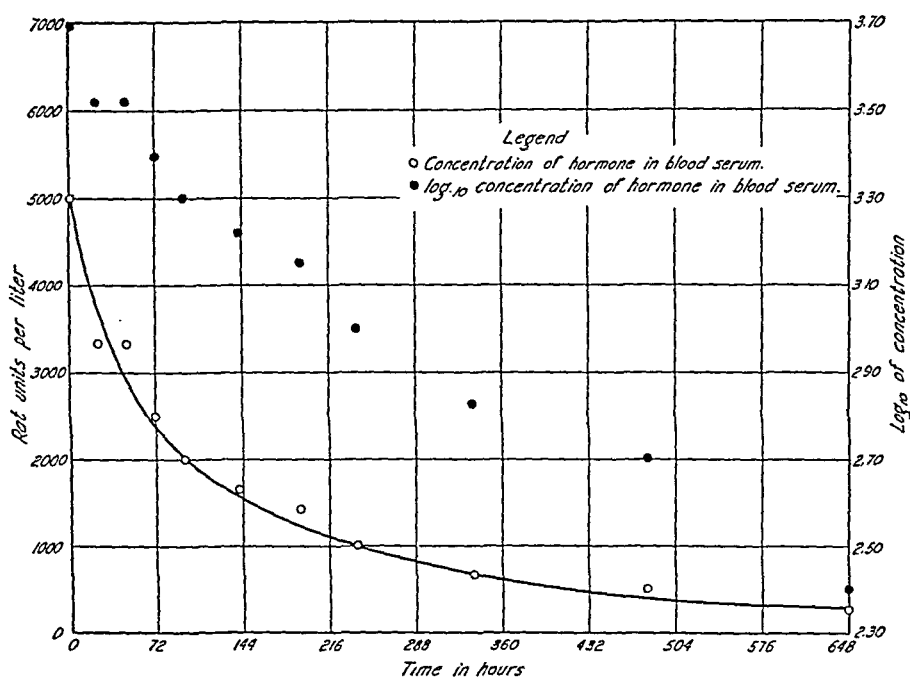


Fig. 2. Disappearance of mare gonadotropic hormone from blood of a gelding following intravenous injection.

hormonal production in the mare, but at a diminishing rate, even over the period when hormone is disappearing absolutely from the body of the mare.

In the rabbit the exact conditions of destruction are harder to define, as the absolute rate of decrease of hormone is very much greater, its concentration in the blood being reduced to $\frac{1}{2}$ every 26 hours. It is possible that, as in the gelding, the initial fall in hormonal concentration is greater than in subsequent decreases, because an inspection of the earlier part of the rabbit curve would indicate a faster disappearance of hormone than observations taken after several days actually show. Assuming the interpretation of a somewhat greater initial destruction of hormone in both rabbit and gelding, the rabbit values after 48 hours and the gelding values after 72 hours give approximate linear logarithmic curves; however, from

the present results we hesitate to dogmatize as to the exact form of these concentration curves.

These experiments confirm the differences already noticed between pregnant mare hormone and prolan. Whereas the latter is rapidly excreted in the urine, the former remains in the blood stream until it is destroyed. This property of the hormone may render it of particular importance therapeutically; the persistence of the hormone for some time in the blood stream allows its action on the gonads to approximate more closely to a continuous one than does that of the readily excretable prolan. This is undoubtedly an explanation of the finding of Cole et al. (1932) that single doses of the hormone are as efficacious as split doses in the rat.

The fact that the body can destroy this hormone throws a new light on hormonal relationships in the mare itself. It is difficult to escape the conclusion that destruction occurs from the first, and probably at much the same rate as we have demonstrated for the gelding. This would indicate that the production of hormone in the pregnant mare is much larger than a study of hormonal concentrations at any one time would indicate. The present experiments indicate that the animal body is capable of metabolising and destroying this hormone promptly, at a rate depending roughly on the concentration of hormone in the blood stream, and on the animal species used. Further, they indicate that the fetus and its membranes are not concerned in the destruction of the hormone in the pregnant mare.

SUMMARY AND CONCLUSIONS

Data are given showing the rate of disappearance of mare gonadotropic hormone from the blood stream of the rabbit and the gelding following administration of large doses of the hormone. The hormonal concentration was reduced approximately one-half every 26 hours in the rabbit and one-half every 6 days in the gelding. The latter rate is definitely greater than in the pregnant mare and indicates that, in the mare, hormone is being produced coincidentally with its destruction. There is no evidence either that the hormone is excreted in the urine or feces of the rabbit or that it is stored in the uterus, spleen, lungs, kidneys, or liver. Thus it is concluded that the hormone is destroyed within the animal body. As the rates of disappearance are similar in castrated and non-castrated rabbits it is improbable that the gonads play a significant rôle in its destruction.

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RESERVE STORE OF HEMOGLOBIN PRODUCING SUBSTANCES IN GROWING DOGS AS INFLUENCED BY DIET

F. S. ROBSCHUIT-ROBBINS AND G. H. WHIPPLE

*From the Department of Pathology, School of Medicine and Dentistry, The University
of Rochester, Rochester, New York*

Received for publication February 1, 1935

Early in our anemia work we suspected a reserve store of materials out of which hemoglobin could be produced but this could not be demonstrated by the short anemia experiments which were first used (4). When the long continued anemia due to blood loss in dogs was substituted for the short anemia experiments we soon had evidence of a reserve store out of which the dog could fabricate much hemoglobin. Moreover when a favorable diet such as liver was fed during a 2-week period we observed much new hemoglobin production in the second week of liver feeding but almost as much in the 2 control weeks of bread following the liver diet. If the dog produced 100 grams new hemoglobin as a result of the liver diet we observed that about 40 grams appeared in the after control period of 2 weeks—a “carry over” due to *storage* of hemoglobin building materials in the body which had to be exhausted before the dog was back to its base line level of hemoglobin production due to standard bread feeding. If the favorable diet regimen continued longer could the body store greater amounts of this hemoglobin producing material? The tabulated experiments below indicate that even during growth periods a large *reserve store* of hemoglobin producing materials will accumulate in the body due to favorable diet intake. This reserve store of hemoglobin building material in the dog is always to be considered in interpretation of results. When anemia periods are short obviously it may introduce large factors of error and to be sure of one's figures long control periods should follow the initial bleedings to establish the anemia level and exhaust the reserve store.

METHODS. The general plan of procedure was very simple. As opportunity offered litters of pups born in the animal quarters were vaccinated against distemper. Immediately after weaning at 8 weeks of age the pups were divided into groups and fed the diets indicated. A whole milk powder “Klim” was added to all diets—30 grams per day. The growth curves were all normal and pups were active and healthy. Particular attention was given to the feeding of these animals and often individual dogs were fed in separate pens to insure adequate food intake for each dog; however

the weight curve is a fairly safe index. When the diet consisted of salmon bread and some meat product, the liver, kidney or meat was put through a meat grinder and then mixed with water and the salmon bread to a hash. This prevents the dog from picking out any particular material which it may prefer. The pups were fed twice daily up to the age of 4 months and once daily thereafter. The special diet was continued for 10 months or up to the age of 1 year when the dogs were ready for the standard bleeding to ascertain the amount of reserve storage of hemoglobin factors. They have attained their mature weight by this time.

Various details relating to ingredients and preparation of the standard salmon bread, care of animals, technique of bleeding and methods have been carefully described (5). The anemia is produced by cautious bleeding and carried to a hemoglobin level about one-third of normal. If this is done carefully the dogs remain perfectly healthy and active and eat their diets completely. Exhaustion of the reserve store of hemoglobin producing factors usually requires 50 to 60 days and this period was used in all animals included in the tables.

EXPERIMENTAL OBSERVATIONS. The standard salmon bread contains wheat flour, potato starch, cane sugar, salmon, canned tomato, cod liver oil, yeast and a salt mixture minus iron, and is an adequate diet for growth or maintenance. This standard salmon bread is not favorable for hemoglobin production in anemia yet it is adequate for the maintenance of hemoglobin production during the growth of pups from weaning (8 weeks) to maturity (1 year of age). There is little storage of hemoglobin building material in the body during salmon bread periods and when we examine table 1 we note the average value for total hemoglobin reserve as 30 grams hemoglobin. The original level of total hemoglobin circulating in the blood is 249 grams or our reserve store is only 12 per cent. Considering the method errors, the activity of the spleen and other unknown variables, we do not lay any stress on this figure. We may say that on a diet of salmon bread the young dog maintains a normal hemoglobin level and perfect health but does not store away in its body much material from which hemoglobin can be fabricated in an emergency.

Method limitations relating to blood volume have been reviewed (1) and we believe the error is greater when the hemoglobin is high and the mixing of red cells and plasma in the smaller vessels is less complete. The plasma volume is reasonably accurate as determined by the brilliant vital red dye method but the red cell volume is calculated on the basis of the hematocrit sample taken from the jugular prior to injection of the dye. Obviously there are possibilities of error here as indicated (1). The total blood volume may be figured as the sum of plasma and red cells and the total circulating hemoglobin is the product of the total volume times the hemoglobin per 1 cc. of blood as given in columns 5 and 9, table 1. As the

dog is made anemic the loss of red cells is made up in large part by an increase in plasma volume but the total blood volume in anemia is decreased 10 to 25 per cent. On the average in the various tables we see that the red

TABLE 1
Salmon bread

DOG NO.	WT. AT BEGIN.	NON-ANEMIC				ANEMIC				Hb TOTAL REMOV.	TOTAL Hb RE- SERVE
		Blood volume		Hb. per 100 cc. blood	Total circu- lating Hb	Blood volume		Hb. per 100 cc. blood	Total circu- lating Hb		
		Plas.	R.B.C.			Plas.	R.B.C.				
	kgm.	cc.	cc.	gm.	gm.	cc.	cc.	gm.	gm.	gm.	gm.
27-231	12.1	497	629	21.8	248	700	157	5.6	49	168	-31
27-236	16.2	550	602	19.2	223	835	209	4.7	50	167	-6
27-238	14.8	610	560	19.2	227	850	217	6.4	68	216	57
30-114	14.8	720	714	17.8	257	894	239	6.1	69	196	8
30-116	14.6	730	633	17.1	234	812	263	6.1	66	229	61
30-120	16.2	536	683	22.0	269	800	273	5.8	63	210	4
32-5	14.5	690	738	19.3	277	780	254	6.5	69	198	-10
32-6	14.4	640	718	19.2	263	806	326	8.4	97	286	120
33-13	16.2	629	660	19.1	247	941	266	6.2	75	241	69
Av...	14.9	622	660	19.3	249	824	245	6.3	67	212	30

TABLE 2
Mixed kennel diet

DOG NO.	WT. AT BEGIN.	NON-ANEMIC				ANEMIC				Hb TOTAL REMOV.	TOTAL Hb RE-SERVE
		Blood volume		Hb per 100 cc. blood	Total circulating Hb	Blood volume		Hb per 100 cc. blood	Total circulating Hb		
		Plas.	R.B.C.			Plas.	R.B.C.				
	kgm.	cc.	cc.	gm.	gm.	cc.	cc.	gm.	gm.	gm.	gm.
24-59	16.9	654	926	17.4	277	946	314	6.5	83	227	33
25-23	15.8	578	648	16.7	206	760	236	6.8	68	165	27
26-18	14.6	776	535	15.5	205	939	255	6.1	73	261	129
26-102	12.9	612	532	17.8	205	807	201	5.8	59	234	88
26-164	12.8	562	574	18.8	216	758	215	6.6	65	187	36
29-65	12.0	520	750	23.0	295	740	184	6.5	60	209	-26
29-66	13.4	391	540	22.4	209	836	221	6.2	66	230	87
29-67	15.7	745	841	20.1	323	929	220	5.7	65	232	-26
29-68	14.4	640	848	21.2	319	926	200	5.4	61	247	-11
Av...	14.4	609	688	19.2	251	849	227	6.2	67	221	37

cell volume decreases about 400 cc. while the plasma volume increases about 200 cc. or the *total blood volume* in the anemic state is about 200 cc. less than in the non-anemic or normal state.

The *total hemoglobin reserve* is readily calculated from the figures given

in the tables. The total anemic circulating hemoglobin (67 gm.) plus the total hemoglobin removed by bleeding (212 gm.) (table 1) averages 279 grams which exceeds the original level of circulating hemoglobin in the non-anemic state (249 gm.) by 30 grams. The *reserve figure* is 30 grams hemoglobin. We may choose to say that some of this 30 grams hemoglobin was held in the spleen and set free as the anemia progressed. It has been shown that there is no reserve of mature red cells in the bone marrow under such conditions (2).

TABLE 3
Kidney and salmon bread, equal parts

DOG NO.	WT. AT BEGIN.	NON-ANEMIC				ANEMIC				Hb TOTAL REMOV.	TOTAL Hb RE-SERVE
		Blood volume		Hb per 100 cc. blood	Total circulating Hb	Blood volume		Hb per 100 cc. blood	Total circulating Hb		
		Plas.	R.B.C.			Plas.	R.B.C.				
	kgm.	cc.	cc.	gm.	gm.	cc.	cc.	gm.	gm.	gm.	gm.
27-239	11.4	541	754	21.4	280	724	234	5.2	50	281	51
27-240	12.3	500	898	23.3	329	845	225	6.1	66	238	-25
27-241	12.3	482	654	21.7	249	714	238	7.2	69	235	55
Av...	12.0	508	768	22.1	286	761	232	6.2	62	251	27

TABLE 4
Skeletal muscle and salmon bread, equal parts

DOG NO.	WT. AT BEGIN.	NON-ANEMIC				ANEMIC				Hb TOTAL REMOV.	TOTAL Hb RE-SERVE
		Blood volume		Hb per 100 cc. blood	Total circulating Hb	Blood volume		Hb per 100 cc. blood	Total circulating Hb		
		Plas.	R.B.C.			Plas.	R.B.C.				
	kgm.	cc.	cc.	gm.	gm.	cc.	cc.	gm.	gm.	gm.	gm.
30-117	15.3	688	762	19.3	283	986	290	6.5	83	330	130
30-118	13.4	531	546	18.5	200	797	251	7.0	74	247	121
30-121	14.7	744	766	18.5	281	886	274	6.8	79	305	103
32-1	13.2	600	642	20.1	252	725	234	6.9	66	217	31
32-3	12.8	610	580	18.6	223	762	224	6.6	66	228	71
Av...	13.9	634	659	19.0	248	831	255	6.8	74	265	91

It is obvious from the weights that these dogs are of a uniform type. They were bred from our anemia stock. These animals are predominantly white bull dog but strains of coach and terrier are represented. Many of the dogs in table 1 were litter mates as indicated by the numbers. Even in the same litter we find considerable differences in the capacity of individual dogs to make hemoglobin on standard diets. This has been touched upon in a study of the marrow (2) in many of the anemia colony dogs.

Table 2 shows results very much like table 1 and the total hemoglobin

reserve averages only 37 grams. The kennel diet therefore is adequate for growth and maintenance of the normal hemoglobin level but does not supply a large surplus for reserve purposes. The mixed kennel diet is made up of mixed hospital table scraps and contains much bread, potato and other vegetable material with variable amounts of meat, bones and butter.

Table 3 shows that kidney added to the salmon bread in equal amounts has little effect upon the reserve store of hemoglobin building factors. The average figure for the total reserve store is 27 grams hemoglobin and this is not beyond possible method error. The series is small and no conclusions should be drawn. All three dogs were litter mates and on the average are somewhat smaller than other series. There are individual differences to be noted, especially the high hemoglobin values in the non-anemic period (dog 27-240).

TABLE 5
Liver and salmon bread, equal parts

DOG NO.	WT. AT BEGIN.	NON-ANEMIC				ANEMIC				Hb TOTAL REMOV.	TOTAL Hb RE- SERVE
		Blood volume		Hb per 100 cc. blood	Total circu- lating Hb	Blood volume		Hb per 100 cc. blood	Total circu- lating Hb		
		Plas.	R.B.C.			Plas.	R.B.C.				
	kgm.	cc.	cc.	gm.	gm.	cc.	cc.	gm.	gm.	gm.	gm.
25-97	15.3	735	864	16.8	272	927	356	6.8	88	308	124
27-233	11.5	510	500	19.0	195	760	188	6.5	63	233	101
27-234	14.0	724	625	17.4	237	836	229	6.6	71	307	141
27-235	14.4	512	800	24.6	326	836	206	6.1	64	297	35
30-115	13.0	624	558	16.3	194	809	241	6.1	65	349	220
30-119	13.3	484	381	15.5	135	696	187	5.3	47	233	145
32-2	15.1	765	736	18.2	276	988	182	5.0	59	276	59
32-4	14.1	530	682	20.7	251	800	267	6.9	74	273	96
Av...	13.8	611	643	18.6	236	831	232	6.2	66	285	115

Table 4 shows that skeletal muscle (beef) added to the salmon bread in equal amounts has a significant effect upon the reserve store of hemoglobin building material. The average figure for the total hemoglobin reserve store is 91 grams which is far beyond method or physiological variables. Beef muscle is not as efficient for rapid hemoglobin regeneration as kidney but for the storage of this hemoglobin reserve it seems to be distinctly superior.

Table 5 as might be expected shows that *liver* is the optimum diet factor to bring about a large reserve store of hemoglobin building material in the young animal. The total store of hemoglobin building material represents 115 grams or about one half the original hemoglobin in circulation in the non-anemic state. This represents more than 380 cc. of packed red cells or 760 cc. of normal dog blood and we cannot imagine that the spleen

or bone marrow can account for more than a small fraction of this large reserve store. One may speculate as to the form and location of this store. We suspect that a large part is stored in the liver, but work in progress may throw light on this point. The protein reserve which is related to the plasma protein output (3) may also be concerned or there may be a common protein reserve store which is mobilized in emergencies either toward hemoglobin or plasma protein.

SUMMARY

In normal growing dogs from weaning (8 weeks of age) to the adult state (1 year of age) there is ample hemoglobin building material for maintenance but there is little reserve storage of these hemoglobin producing substances (30 gm. hemoglobin) when the diet consists of standard salmon bread.

When skeletal muscle (beef) is added to the basal ration there is demonstrable a considerable reserve store of hemoglobin producing material—91 grams hemoglobin.

With liver feeding during this growth period the dog will store away a large supply of material out of which new hemoglobin can be produced in an emergency—115 grams hemoglobin.

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THE CHEMICAL MEDIATION OF SYMPATHETIC VASODILATOR NERVE IMPULSES

A. ROSENBLUETH AND W. B. CANNON

From the Laboratories of Physiology in the Harvard Medical School

Received for publication January 30, 1935

Dale and Feldberg (1934) have recently emphasized the probability that, although sympathetic nerve impulses are usually mediated by the adrenaline-like sympathin, some may act through liberation of acetylcholine. Dale (1934) suggests that the former be called adrenergic and the latter cholinergic.

That in appropriate experimental conditions adrenaline may evoke vasodilatation is well known. The vasodilator effects of acetylcholine likewise are well known. It appeared to us important to determine whether the widely distributed sympathetic dilators in the cat and dog are adrenergic, or cholinergic, or both, and whether adrenaline exerts its depressor effects directly or through liberation of acetylcholine.

The only tests available for distinguishing between adrenaline and acetylcholine in the amounts which are liberated by stimulation of autonomic nerves are pharmacological. We therefore studied the effects of ergotoxine and atropine, cocaine or eserine, on the blood-pressure changes caused by acetylcholine, adrenaline and the stimulation of sympathetic nerves in the cat and dog. The rabbit also was examined because of its peculiar reactions to adrenaline—no depressor effects from small doses of the hormone and no “reversal” of the pressor into depressor effects by ergotoxine (Dale, 1906).

METHOD. The animals were anesthetized with either dial or urethane or they were rendered spinal by destruction of the brain while under ether anesthesia. The blood pressure was recorded from a carotid or a femoral artery by means of a mercury or a Hürthle manometer. The drugs were injected intravenously. The nerves (usually the lower abdominal chains, crushed or severed centrally) were stimulated with a Harvard inductorium through buried shielded electrodes.

RESULTS. A. *Ergotoxine in the cat.* The depressor effect of adrenaline after ergotoxine is a well-established fact. Stimulation of sympathetic nerves, e.g., the lower abdominal chains, which previously induced a rise of blood pressure, will then evoke a fall which is usually succeeded by a rise. The initial fall has been interpreted as due to the effects of sympathetic

dilators (Dale, 1906; Cannon and Rosenblueth, 1933). In numerous experiments we have invariably found that after ergotoxine acetylcholine is quite effective in evoking depressor responses.

Because ergotoxine consistently conditions a depressor response to stimulation of sympathetic fibers distributing to peripheral structures (skin and muscles), it was routinely injected and the other pharmacological tests, to be described in the succeeding sections, were thereafter applied.

B. Cocaine in the cat. The depressor effects of adrenine (commercial adrenalin) after ergotoxine are augmented by cocaine in doses of 8 mgm. per kgm. (fig. 1). The responses to acetylcholine are not much nor con-

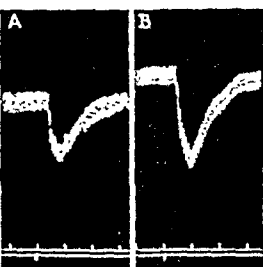


Fig. 1



Fig. 2

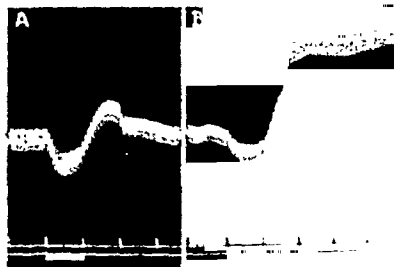


Fig. 3

Fig. 1. Augmenting effect of cocaine on the fall of blood pressure evoked by adrenine after ergotoxine. Spinal cat under curare. Ergotoxine (4 mgm. per kgm.). At the signals 0.2 cc. adrenine (1:100,000) injected intravenously. Time intervals in this and the succeeding figures: 30 seconds.

A. Before cocaine.

B. After the injection of cocaine (8 mgm. per kgm.).

Fig. 2. Negligible effects of cocaine on the fall of blood pressure elicited by acetylcholine. Cat under dial anesthesia. Ergotoxine (4 mgm. per kgm.). At the signals 0.1 cc. acetylcholine bromide, 1:1,000,000.

A. Before cocaine.

B. After the injection of cocaine (8 mgm. per kgm.).

Fig. 3. Effects of cocaine on the responses to stimulation of the lower abdominal sympathetic chains after ergotoxine (2 mgm. per kgm.). Cat under dial anesthesia. At the signals the nerves were stimulated with the same intensity.

A. Before cocaine.

B. After the injection of cocaine (8 mgm. per kgm.).

sistently altered by cocaine (fig. 2). As regards the action of cocaine on the complex of responses to stimulation of the lower abdominal sympathetic chains after ergotoxine, the initial fall is either not changed or may be decreased, while the subsequent rise is usually augmented (fig. 3).

C. Atropine in the cat. Atropine (1 mgm. per kgm.) does not prevent the depressor effect of adrenine after ergotoxine (fig. 4). On the other hand, the influence of acetylcholine on the blood pressure, even when given in doses as large as 0.001 mgm., is wholly abolished by atropine.

The effect of atropine on the results of sympathetic nerve stimulation are complex and depend largely on the anesthetic employed. If the cat is under dial anesthesia the dose of atropine mentioned above merely decreases the initial fall, leaving the delayed rise unimpaired (fig. 5). In decapitate animals, to which curare and ergotoxine have been administered, atropine may eliminate entirely the initial drop of pressure, and the delayed rise may be replaced by a slight prolonged fall (fig. 6) with subsequent recovery. This delayed fall was especially marked when the hypogastric nerves were stimulated.

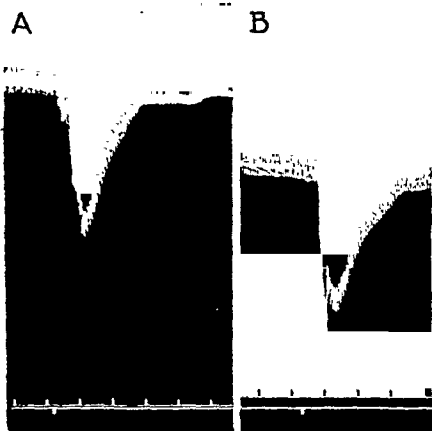


Fig. 4

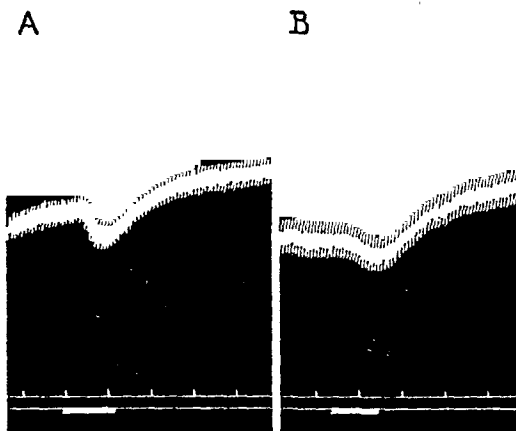


Fig. 5

Fig. 4. Negative effects of atropine on the fall of blood pressure elicited by adrenaline after ergotoxine. Spinal cat under curare. Ergotoxine (4 mgm. per kgm.). At the signals 0.1 cc. adrenaline, 1:100,000.

A. Before atropine.

B. After the injection of atropine (1 mgm. per kgm.).

Fig. 5. Persistence after atropine of the initial fall of blood pressure evoked by stimulation of the lower abdominal sympathetic chains in a cat under dial anesthesia and injected with ergotoxine and cocaine. At the signals the nerves were stimulated with the same intensity.

A. Before atropine.

B. After the injection of atropine (1.2 mgm. per kgm.).

D. *Eserine in the cat.* This drug (0.1 to 0.2 mgm. per kgm.) has no influence on the vascular responses to adrenaline. The marked augmenting action of eserine on the effects of acetylcholine is too well known to require any illustration. The initial fall of arterial pressure on stimulation of the lower abdominal sympathetic chains after ergotoxine may be uninfluenced or definitely enhanced by eserine; the delayed rise is usually unaffected (see fig. 7 for the similar phenomenon in the dog).

E. *Curarc.* Since this drug was administered to the spinal cats in order

to avoid the convulsant effects of ergotoxine and eserine, it was important to determine its influence on the responses studied. This influence was found to be practically negligible as affecting the results of injecting adrenaline or stimulating the nerves, while there was usually a definite decrease in the drop of blood pressure elicited by acetylcholine.

F. The dog. The action of ergotoxine and cocaine in dogs was similar to that noted in cats. The effects of eserine in enhancing the responses

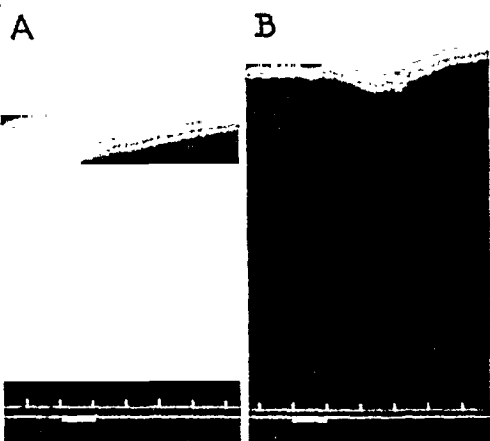


Fig. 6

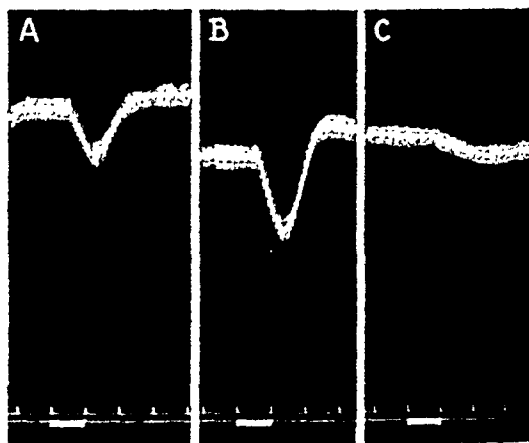


Fig. 7

Fig. 6. Abolition by atropine of the initial fall of blood pressure evoked by stimulation of the lower abdominal sympathetic chains in a spinal cat injected with curare, ergotoxine and cocaine. At the signals the nerves were stimulated with the same intensity.

A. Before atropine.

B. After injection of atropine (1 mgm. per kgm.).

Fig. 7. Increase by eserine and abolition by atropine of the initial fall of blood pressure evoked by stimulation of the lower abdominal sympathetic chains in a dog under dial anesthesia and injected with ergotoxine. At the signals the nerves were stimulated with the intensities noted below.

A. Before eserine and atropine. Coil distance 8 cm.

B. After injection of eserine (0.1 mgm. per kgm.). Coil distance 8 cm.

C. After injection of atropine (1 mgm. per kgm.). Coil distance 7 cm.

to acetylcholine are especially striking in the dog, since before eserine relatively large doses of acetylcholine are necessary to obtain any depressor response. For example, in one instance, after ergotoxine, 0.5 cc. acetylcholine (1:100,000) caused a fall of blood pressure from 144 to 136 mm. Hg; after eserine (0.1 mgm. per kgm.) 0.05 cc. acetylcholine in the same dilution reduced the pressure from 130 mm. Hg to 70. Eserine increases also the initial fall attending nerve stimulation (fig. 7A and B).

Atropine in a dose of 1 mgm. per kgm., which does not abolish the initial

fall of blood pressure on stimulation of the lower abdominal sympathetic chains in cats under dial and given ergotoxine (section C, fig. 5), does readily abolish this initial depressor effect in dogs under dial (fig. 7C).

G. *The rabbit.* Ergotoxine induces a fall of blood pressure in rabbits under urethane, instead of the rise invariably obtained in cats under various anesthetics. As mentioned in the introduction, Dale (1906) showed that after ergotoxine adrenine does not induce a fall of blood pressure in rabbits as it does in cats. We readily confirmed this fact. Stimulation of the lower abdominal sympathetic chains in the same experimental conditions failed invariably to elicit any fall of blood pressure, whether initial or delayed. Administration of cocaine did not modify the negative results.

H. *Influence of the anesthetic.* The difference of effects of atropine when injected into spinal cats or into cats anesthetized with dial has been mentioned (section C). A further difference between these two preparations is that ergotoxine is more efficient in paralyzing pressor responses to adrenine or to nerve stimulation in spinal, curarized cats than in cats under dial (cf. Cannon and Rosenblueth, 1933). In the present experiments this difference was again noted, especially in the delayed effects of stimulation of the lower abdominal sympathetic chains. In animals under dial this delayed effect was usually a rise and atropine had little influence on it. In the spinal animals, on the other hand, there was frequently no delayed rise after ergotoxine, the preliminary fall being prolonged into a slow gradual recovery, and the delayed fall after atropine was particularly prominent and easy to obtain.

DISCUSSION. The object of these experiments was to obtain information regarding the adrenergic or cholinergic nature of sympathetic dilators. Pharmacological tests had to be employed, as previously remarked, because they alone were available. We feel, however, that only tentative conclusions may be derived from the data presented, because these data seem to us to illustrate how untrustworthy pharmacological evidence may be and how cautiously we should proceed before drawing any physiological inferences therefrom.

If cats and dogs had been observed solely *under dial anesthesia* a striking difference would have appeared as regards the action of atropine (sections C and F). Since the same doses of this drug (1 mgm. per kgm.) abolish the initial fall of blood pressure resulting from sympathetic stimulation after ergotoxine in the dog, and not in the cat, one might conclude that there is a cholinergic dilator component in the abdominal sympathetic of the dog which is absent or minimal in the cat. When, however, the same dose of atropine is given to spinal cats, the initial fall disappears entirely (section C), showing that the difference may be only quantitative and due to specific variation in susceptibility to atropine in the two experimental conditions, and not to differences in the chemical mediation of the nerves involved.

Further, when the inference is drawn that a response is mediated by acetylcholine because it is impaired or abolished by atropine, the tacit assumption is made that atropine is without influence on the effects of adrenaline or sympathin. Such an assumption may be incorrect (cf. Magnus, 1908 and Gasser, 1930).

From the data presented, however, certain conclusions appear to be justified. The depressor action of adrenaline is probably a direct effect, and not due to liberation of acetylcholine. In support of this view is the increase of the responses by cocaine (fig. 1), the persistence after atropine (fig. 4) which abolishes the action of acetylcholine, and the lack of augmenting action of eserine (see section D). The effect, furthermore, is probably specific (i.e., adrenaline acts on specific vessels which possess an adequate receptive mechanism) and not a generalized action on any vessels. In support of this statement is the absence of depressor effects in rabbits under conditions which in cats and dogs invariably yield a fall of blood pressure.

These views concerning the depressor action of adrenaline, together with an application of Elliott's law, lead us to expect that certain of the vasodilator sympathetic fibers in the cat and dog should be adrenergic. We should also expect no adrenergic dilators in the rabbit. That the blood pressure does not fall on stimulation of the lower abdominal sympathetic chains in the rabbit, given ergotoxine (section G), confirms this expectation. It further leads to the conclusion that cholinergic sympathetic dilators are likewise absent—i.e., that there are *no* sympathetic dilators to the hind limbs or tail in the rabbit.

The initial fall on sympathetic stimulation in the dog and the spinal cat, after ergotoxine, is abolished by atropine (figs. 6 and 7C), is not consistently modified by cocaine (fig. 2), and is increased by eserine (fig. 7B). It seems probable, therefore, that there is a cholinergic component partly responsible for the effect. Bülbring and Burn (1934), working with perfused limbs of cats and dogs, report that in the dog eserine increases the dilator effects of sympathetic stimulation and atropine abolishes them, whereas in the cat the responses are unaffected by either drug. They conclude that cholinergic sympathetic dilators are present in the dog and absent in the cat. This discrepancy between Bülbring and Burn's results and inferences and our own may be due to the variability of the preparation which they employed to demonstrate sympathetic dilators. Indeed, they report irregular results from sympathetic stimulation in the perfused limbs, constriction instead of dilatation being often the response. They further had to use adrenaline in their perfusing fluid in order to permit a dilatation to occur, and, as pointed out above (see p. 34), atropine may not be without action on the responses which involve adrenaline. Finally, it would appear from our data that atropine is relatively more effective in dogs than in cats in block-

ing sympathetic dilators (sections C and F). The differences observed by Bülbring and Burn may then again be merely quantitative, not qualitative.

If cholinergic sympathetic dilators are present in the cat and dog the question again arises whether there are any adrenergic ones, as Elliott's law would lead us to expect. We believe that the instances of a *delayed* fall of blood pressure (see figs. 6B and 7C, and section C) favor an affirmative answer to this question. It must not be forgotten that, as we have previously noted (Cannon and Rosenblueth, 1933), the paralyzing effects of ergotoxine on constrictor sympathetic impulses are relative, dependent on the dose of ergotoxine and on the anesthetic employed (cf. section H). When we stimulate, after ergotoxine, a mixed sympathetic trunk, containing both constrictors and dilators, the constrictors may still have some immediate local effect—smaller, however, than before the ergotoxine. The changes of blood pressure will then depend on the relative importance of the constriction and dilatation. When the cholinergic dilators are present, before atropine, an immediate fall occurs. Indeed, the briefness of this fall (figs. 3, 5 and 7) is in harmony with the rapid destruction of acetylcholine and the correspondingly rapid disappearance of cholinergic responses. When the cholinergic dilators have been blocked by atropine, the blood-pressure changes will depend on the balance of adrenergic constriction and dilatation, local and general (sympathins E and I). The fact that falls are more frequent and significant with larger doses of ergotoxine and in spinal cats than in cats under dial (section H) is in favor of the presence of adrenergic dilators.

Since, however, atropine not only may abolish the initial, presumably cholinergic, fall of blood pressure but also frequently converts a delayed rise into a delayed fall, we are finally led to conclude that atropine either augments the effects of sympathin I, or impairs the responses to sympathin E, or both.

SUMMARY

In anesthetized cats, dogs and rabbits, injected with ergotoxine, the changes of blood pressure evoked by adrenaline, acetylcholine and stimulation of the lower abdominal sympathetic chains were recorded before and after cocaine, eserine and atropine.

In cats and dogs cocaine increases the fall caused by adrenaline (fig. 1) and the delayed rise elicited by sympathetic stimulation (fig. 3); eserine increases the initial fall elicited by sympathetic stimulation (fig. 7B); and atropine diminishes (fig. 5) or abolishes (figs. 6 and 7C) this initial fall and may change the delayed rise into a slight delayed fall (figs. 6 and 7C). In rabbits stimulation of the lower abdominal sympathetic chains does not evoke a fall of blood pressure after ergotoxine.

It is concluded that the effects of adrenaline are specific and not mediated by acetylcholine (p. 38); that the sympathetic contains some cholinergic (p. 38) and some adrenergic (p. 38) vasodilators in cats and dogs, and no vasodilators at all in the rabbit (p. 38).

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EVIDENCE FOR A POTASSIUM SHIFT FROM PLASMA TO MUSCLES IN RESPONSE TO AN INCREASED CARBON DIOXIDE TENSION

W. O. FENN AND DORIS M. COBB

From the Department of Physiology, School of Medicine and Dentistry, The University of Rochester, Rochester, N. Y.

Received for publication February 5, 1934

Complete osmotic equilibrium can be maintained across a water permeable membrane by one of three methods: 1, complete impermeability to electrolytes; 2, complete impermeability to anions or to cations; 3, maintenance of hydrostatic pressure to balance the osmotic pressure. There are three membranes in the body which are of special importance from this point of view: the membrane of the red blood corpuscle, that of the tissue cells, and that of the capillary walls. Of these three, the last is permeable to all electrolytes and must maintain its complete equilibrium by the maintenance of a capillary blood pressure to balance the colloid osmotic pressure of the blood. Osmotic equilibrium across the red blood corpuscle membrane is maintained by its impermeability to cations. There is evidence in muscle cells that the equilibrium is maintained by complete impermeability to anions.

Across the red blood corpuscle membrane there is a shift of chloride from plasma to corpuscles when the carbon dioxide tension is increased. If the muscle cell is permeable to cations rather than to anions, a similar increase in carbon dioxide tension should result, not in a chloride shift, but in a potassium shift, since apparently the potassium is the only cation normally present which can penetrate (except the H^+ ion). The direction of this shift will naturally depend on the relative buffering capacities of the cells and plasma. If an increase in carbon dioxide tension causes a greater increase in acidity in the cells than in the plasma, then potassium will tend to shift from the plasma to the muscles in order to neutralize this acidity. If the buffering power of the plasma is artificially diminished by substituting Ringer's solution, then the same increase in carbon dioxide tension should increase the acidity of the solution more than the acidity of the cells and cause potassium to go from the cells to the solution.¹ Since the muscles

¹ In our former paper (1934) we erroneously predicted a movement of potassium from cells to plasma as a result of increased carbon dioxide tension because of failure to realize that blood is better buffered than muscle.

are impermeable to anions no chloride shift would be expected between the blood and the cells.

As far as the red blood corpuscles are concerned, this theory may be regarded as well established. For the muscle cell it may probably be regarded as merely an hypothesis, although a good deal of evidence has been accumulated in its favor. The following experiments were designed to add to this evidence and they serve to show that in the sartorius muscle immersed in its own blood an increase of carbon dioxide tension causes potassium to move from the plasma to the muscles as expected, while the reverse is found in Ringer's solution. They show further that there is no chloride shift to be detected.

METHOD. The experiments were performed on frogs (*Rana pipiens*). The brain was crushed with a strong hemostat, the heart exposed and the blood drawn directly from the aorta into a syringe containing about 1 mgm. of heparin. Between 1 and 2 cc. of blood could be obtained from each frog. Sartorius muscles were dissected out and immersed in this blood equilibrated at different carbon dioxide tensions. In some cases, the blood from several frogs was pooled and the muscles were equilibrated in a 25 cc. flask which was rotated slowly in a horizontal position in the water bath at 22°C. In other experiments the blood from one frog was divided equally between two test tubes (by counting drops from the syringe) and two sartorius muscles were immersed, one in each test tube. The tubes were agitated gently in the water bath to prevent settling of the blood corpuscles. After a period, usually 5 hours in duration, the muscles were removed, weighed again on a torsion balance and set aside for analysis. The blood was centrifuged without exposure to air and the plasma was analyzed either for chloride or for bicarbonate.

Potassium was analyzed by the method of Shohl and Bennett (1928) as previously described (Fenn and Cobb, 1934) with one modification introduced by Dr. J. I. Thaler in this laboratory (unpublished). This consisted in separating out the precipitate of potassium chloroplatinate by centrifuge instead of by filter since great difficulty was experienced in finding reliable filters. This procedure did not seem to give the occasional large errors which caused Shohl and Bennett to discard the use of the centrifuge. On the contrary, in our experience, the new method is to be preferred because it eliminates occasional large errors met with in using filters and obviates the necessity of testing the filter each day on a known solution. The methods used for chloride analyses have been previously described (Fenn, Cobb and Marsh, 1934).

RESULTS. *Control analyses.* A series of control analyses of matched muscles are collected in table 1. The tibialis anticus longus and the semitendinosus were included because they have about the same weight as the sartorius muscle. The muscles were moistened with Ringer's solution

during dissection, then dipped momentarily in that solution, blotted, weighed on a torsion balance and analyzed. The average difference between the two analyses made on a pair of muscles was 2 per cent, and the probable error in analyzing matched muscles is 0.115 m.-eq. per cent or 1.4 per cent. These analyses with the new method are somewhat better than those previously reported (Fenn and Cobb, 1934).

Potassium changes during immersion in frog blood. When frog sartorius muscles are dissected out and immersed for 5 hours in Ringer phosphate solution at 22°C. and pH 7.2 they lose about 10 per cent or more of their potassium and this loss increases with increase in acidity and vice versa (Fenn and Cobb, 1934). Believing that this loss of potassium was due to

TABLE 1
Control series
Potassium contents of matched muscles
(Milli-equivalents per 100 grams of muscle)

MUSCLE	RIGHT	LEFT	DIFFERENCE
Sartorius	7.88	8.08	+0.20
	8.24	8.28	+0.04
	7.78	7.98	+0.20
	8.72	8.62	-0.10
Semitendinosus	7.88	8.16	+0.28
	7.93	7.85	-0.08
	7.80	7.70	-0.10
	8.03	7.85	-0.18
Tibialis anticus longus	7.44	7.19	-0.25
	7.68	7.80	+0.12
	7.88	7.72	-0.16
Average.....	7.933	7.930	-0.003

the abnormality of the Ringer's solution compared to frog blood we anticipated that if the muscles were immersed in the blood of the same animal equilibrated with carbon dioxide at normal tensions (about 5 per cent) there would be complete potassium equilibrium. We also anticipated that if the carbon dioxide tension were further increased the increase in acidity in the muscle would be greater than the increased acidity in the blood (due to the lower buffering capacity of the muscle) and potassium would move from the blood into the muscle—and vice versa at lower carbon dioxide tensions.

To test this theory the experiments of table 2 were carried out. The experiments consisted in dissecting out the two sartorius muscles of one frog, analyzing one of the muscles immediately as a control and analyzing

the other after it had been immersed for 5 hours in blood equilibrated with different carbon dioxide tensions. The results were contrary to expectations for they showed that at all carbon dioxide tensions from 0.7 to 18.6 per cent there was a loss of potassium from the muscle to the blood amounting on the average to 2.5 per cent of the amount present. The absolute amount lost, 0.26 m.-eq. per cent is 9.3 times the probable error of the mean

TABLE 2

The potassium content of matched muscles before and after immersion for 5 hours at 22°C. in frog blood plus CO₂ and O₂
(M.-eq. per 100 grams initial weight)

EXPERIMENT	CO ₂	Δ WEIGHT	POTASSIUM CONTENT		Δ K
			Before	After	
	<i>per cent</i>	<i>per cent</i>			
1 a	0.7	-0.8	9.00	8.67	-0.33
b	0.7	-4.0	8.52	7.93	-0.59
c	0.7	+1.7	8.80	8.77	-0.03
2 a	1.65	-7.0	8.50	8.30	-0.20
b	1.65	-5.1	9.08	8.98	-0.10
3 a	4.93	-1.0	9.18	8.21	-0.97
b	4.93	-3.0	9.00	8.36	-0.64
c	4.93	-2.1	9.06	8.03	-1.03
4 a	5.0	-2.0	8.87	9.56	+0.69
b	5.0	-7.0	8.82	9.00	+0.18
c	5.0	-9.0	8.75	8.93	+0.18
5 a	9.28	-3.3	8.49	8.82	+0.33
b	9.28	+0.8	9.52	9.02	-0.50
c	9.28	+1.4	9.06	8.85	-0.21
6 a	18.65	0	8.80	7.98	-0.82
b	18.65	0	8.82	8.10	-0.72
c	18.65	-4.0	7.65	8.00	+0.35
Average.....		-2.6	8.82	8.56	-0.26

All muscles weighed between 92 and 140 mgm.

of 17 determinations. In five of the 17 muscles there was a gain in potassium, the amount of the gain being in three cases at least significantly greater than the analytical error. In spite of this evidence of variability due to uncontrolled factors, it must be concluded that under the conditions of these experiments any tendency of the muscles to gain potassium because of high carbon dioxide tension is more than balanced by a tendency to lose potassium due to other factors such as injury to the fibres. Whatever

this loss may be due to it is only about one-quarter as great when the muscles are immersed in blood as when they are immersed in Ringer's solution.

In a further attempt to elucidate the rôle of carbon dioxide in the potassium equilibrium, we carried out the experiments listed in table 3, the object being to soak both muscles in blood, usually at 1 per cent carbon dioxide tension, for a short preliminary period in order to "wash away" any

TABLE 3
Potassium contents of paired muscles and nerves

EXPERIMENT	PRELIMINARY PERIOD		EXPERIMENTAL PERIOD		Δ WEIGHT		POTASSIUM CONTENT		Δ K
	Time	CO ₂	Time	CO ₂	Control	Experimental	Control	Experimental	
Muscle									
	hrs.	per cent	hrs.	per cent	per cent		m.-eq. per 100 gm. initial wt.		
1	0.5	9.3	5	9.3	-3.6	+2.4	7.57	8.11	+0.54
2	0.5	9.3	5	9.3	-2.9	0	8.24	8.44	+0.20
3	0.5	9.3	5	9.3	-1.0	-1.0	8.57	8.59	+0.02
4	2.7	1.0	3.3	9.3	+4.0	+8.6	8.14	8.22	+0.08
5	2.6	1.0	3.3	9.3	+0.8	+3.4	6.98	7.88	+0.90
6	2.5	1.0	3.3	9.3	+4.3	+1.9	7.34	7.11	-0.23
7	2.6	1.0	4.2	17.2	-3.5	-7.1	8.00	8.34	+0.34
8	3.3	1.0	3.1	26	0	+7.1	8.52	8.96	+0.44
9	3.2	1.0	3.3	26	-4.2	-0.8	8.24	7.37	-0.87
10	3.0	1.0	3.1	26	-2.2	-5.2	8.72	8.28	-0.44
Average.....							8.03	8.13	+0.10
Nerve									
11	1.8	1.0	3.2	9.3	-1.0	+8.5	3.55	3.43	-0.12
12	1.5	1.0	4.2	9.3	-10.4	-13.5	2.92	3.50	+0.58
13	2.7	1.0	3.3	9.3	-7.6	-4.0	3.02	3.63	+0.61

Both control and experimental muscles and nerves were immersed in blood-CO₂ for a preliminary period after which the control muscle was weighed and analyzed. The experimental muscle or nerve was then immersed for an experimental period in blood-CO₂, then weighed and analyzed. Muscles weighed 85 to 135 mgm. and nerves 89 to 101 mgm.

potassium due to injury, and to study in a subsequent experimental period the movements of potassium under the influence of different carbon dioxide tensions. At the close of this preliminary period the control muscle was analyzed for potassium and the carbon dioxide tension was increased for the experimental period after which the experimental muscle was also analyzed. Three experiments of this sort were also carried out on nerves, the sciatic nerves from three frogs being used for each experiment.

The results of these experiments, listed in table 3, are somewhat more encouraging. As shown in the last column of table 3, all but three of the ten muscles and two out of three of the nerves showed a gain of potassium during the experimental period. The average gain for the muscles was 0.1 m.-eq. per cent which is approximately three times the probable error of the mean of ten such observations.

In studying the time course of potassium intake by muscles in alkaline Ringer's solution of high potassium content we have found (1934) that an initial gain is followed by a terminal loss of potassium. In interpreting the results of table 3 it may be supposed that the three muscles which showed a loss of potassium had reached the beginning of this terminal stage while most of the others were still near the peak of the intake. It is to be supposed also that at lower carbon dioxide tensions this intake of potassium would not occur. On this basis the results may be taken to indicate that high carbon dioxide tension causes a potassium shift to the cells. The same interpretation may also be applied to the three nerve experiments. The error of the analyses is probably larger here, however, for the absolute amounts of potassium to be measured are less; this probably explains the small loss in one experiment, the gains in the other two cases being much larger.

We do not, however, regard the data of table 3 as a perfectly satisfactory proof of a potassium shift to the cells caused by carbon dioxide. Aside from the smallness of the average gain observed it would be necessary to show also that at low carbon dioxide tensions under otherwise similar conditions there would be no such intake of potassium. Instead of attempting to prove this point we have undertaken a more direct proof by comparing the potassium content of matched muscles immersed in blood for 5 hours at 22°C., one at low and the other at high carbon dioxide tensions. Any factors which cause a loss of potassium due to the disturbance of the normal equilibrium *in vivo* are then equal in the two cases and any difference in potassium content after the period of immersion in the blood must be attributed to the effect of carbon dioxide.

Muscle potassium under high and low carbon dioxide tensions. The results of these experiments are shown in table 4. Two similar experiments on nerve (3 pairs for each experiment) as well as one experiment with 3 pairs of muscles in Ringer's solution instead of blood are also included. The differences in potassium content of the muscles or nerves in the two solutions are shown in the last column, a plus sign indicating more potassium in the muscle or nerve in high carbon dioxide. In 17 out of 20 muscles in blood and in both nerve experiments, the high carbon dioxide resulted in a higher potassium content. The average gain in potassium due to carbon dioxide in the muscles in blood was 0.5 m.-eq. per cent. This is a 6 per cent difference between the two muscles and is 20 times the probable error of the mean of 20 determinations. In other words high carbon

TABLE 4

The effect of carbon dioxide on the potassium equilibrium of muscle and nerve after immersion for 5 hours at 22°C.

EXPERIMENT	CO ₂ TENSION		Δ OF WEIGHT		K CONTENT		K EXCESS DUE TO CO ₂
	a	b	a	b	a	b	b - a
Muscle in blood							
	per cent	per cent	per cent	per cent	m.-eq. per 100 gm. initial wt.		
1	1.01	9.28	-6.0	-3.8	6.93	8.16	+1.23
			-0.9	+5.1	7.57	8.32	+0.75
			-5.6	+0.8	6.70	8.49	+1.79
2	4.93	19.32	-1.9	0	8.59	9.03	+0.44
			0	0	8.92	9.23	+0.31
			-11.3	-6.8	7.93	8.57	+0.64
3	0.89	9.28	-8.5	-7.0	7.32	7.52	+0.20
			-2.6	-1.6	8.29	8.42	+0.13
			-4.1	-4.3	8.16	7.85	-0.31
4	1.6	14.3	-2.7	-2.8	8.24	9.46	+1.22
			-5.4	-3.5	8.08	7.62	-0.46
			-7.4	-5.0	7.78	8.80	+1.02
5	0.7	40.0	-4.2	-4.1	7.66	7.62	-0.04
			-6.1	-0.8	7.51	8.05	+0.54
			0 *	+1.6	7.51	7.87	+0.36
			-2.5	+0.8	7.15	7.80	+0.65
			-1.7*	+8.3	7.77	7.85	+0.08
6	5.0	5.0†	-2.3	-3.1	8.01	8.08	+0.07
			-4.1	-5.2	8.26	8.42	+0.16
			-5.2	-5.5	7.70	8.18	+0.48
Average.....					7.80	8.3	+0.50
Muscle in Ringer's solution							
7	1.0	9.28	+1.9	+1.9	7.75	7.37	-0.38
			-5.6	-5.6	6.98	6.55	-0.43
			-10.0	-13.1	7.19	6.96	-0.23
Average.....					7.31	6.96	-0.35
Nerve in blood							
8	1.6	14.3	-5.1	0	4.37	4.53	+0.16
9	1.2	9.28		-4.0	4.58	5.09	+0.51

* Semitendinosus muscles.

† NaH₂PO₄ added to reduce combined CO₂ from 63 to 42 volumes per cent.

dioxide tension has either caused potassium to move from the plasma to the muscles or at least hindered the diffusion of potassium from muscles to plasma. In experiment 6 the carbon dioxide tension was not varied but instead acid sodium phosphate was added to one-half of the blood, thus reducing its bicarbonate content, when equilibrated with 5 per cent carbon dioxide, from 63 to 42 volumes per cent. In this case also the addition of acid to the blood caused an increase of potassium in the muscles. In experiment 7 Ringer's solution was substituted for blood and the result was exactly opposite, that is, an increase of carbon dioxide accelerates the diffusion of potassium out of the muscles. Further experiments of this type were not repeated because they merely served to confirm our previous experiments on this point (Fenn and Cobb, 1934). The two experiments with frog sciatic nerves in table 4 show that in nerves also, high carbon dioxide tension causes a diffusion of potassium from plasma to nerves. Proof of similarity between nerve and muscle in this respect was lacking in our former work on nerve electrolytes (Fenn, Cobb, Hegnauer and Marsh, 1934).

In the course of these experiments we have usually centrifuged the blood under oil after removal of the muscles and have determined the carbon dioxide content of the "true" plasma at different carbon dioxide tensions by the Van Slyke manometric method. These data are plotted in figure 1 together with similar carbon dioxide dissociation curves for sartorius muscle (from Fenn, 1928) and for our Ringer-phosphate solution. The intermediate position of muscle between blood and Ringer is very evident. When the carbon dioxide tension increases from 1 per cent to 10 per cent, the change in H^+ ion concentration calculated roughly from this graph (if $pK_1 = 6.17$) is 73×10^{-8} in Ringer, 29×10^{-8} in muscle and 9.6×10^{-8} in true plasma. Hence if K^+ diffuses by exchanging with H^+ it should move toward the solution where the increase in H^+ is greatest, i.e., from plasma to muscle and from muscle to Ringer. This is actually what happens, as shown in table 4.

Potassium content and carbon dioxide capacity. If the change in potassium content of the muscles which was produced by high and low carbon dioxide tensions is a true potassium shift it must represent, not a diffusion of potassium as a salt, but an exchange between K^+ and H^+ , or some other cation, just as the chloride shift represents an exchange of anions. If this is the case there must be corresponding changes in the carbon dioxide binding capacity of the muscle. The following measurements of the combined carbon dioxide have shown that this expectation is realized.

In each experiment one sartorius and one ileofibularis muscle were immersed together in 1 cc. or more of frog blood or Ringer equilibrated with 0.5 per cent carbon dioxide, while the matched muscles were in an equal amount of the same solution, equilibrated with 20 per cent carbon dioxide.

After 5 hours at 22°C. the muscles were removed from the blood, dipped momentarily in Ringer's solution, blotted, weighed, then dipped momentarily in unbuffered Ringer's solution to moisten them and put into respirometers containing 12 per cent citric acid in the side arms but no other solutions. Further ionic interchanges were therefore impossible.

Since the normal carbon dioxide tension in frogs is around 5 per cent there is presumably a synthesis of phosphocreatine in 0.5 per cent carbon dioxide and a breakdown in 20 per cent carbon dioxide (Root, 1933). When both sets of muscles are returned to 5 per cent carbon dioxide in the respirometer the reverse processes apparently occur as indicated by the volume changes recorded during the $\frac{1}{2}$ to 2 hours previous to acidification.

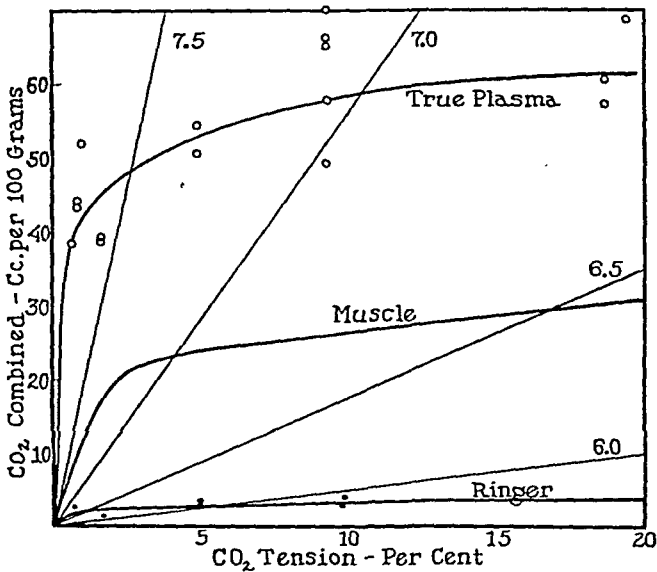


Fig. 1. Carbon dioxide dissociation curves of plasma, muscle and Ringer to show the relative buffering capacities.

The magnitude of these volume changes during equilibration are included in table 5, the minus sign indicating an absorption of carbon dioxide and vice versa. Before tipping the acid we usually waited 2 hours until these changes (presumably of phosphocreatine) were complete. As shown in table 5 the muscles previously in 20 per cent carbon dioxide gave the higher carbon dioxide capacity in blood and the lower carbon dioxide capacity in Ringer. It might be argued that the result in blood was due to the phosphocreatine breakdown in 20 per cent carbon dioxide which had not been completely reversible but this should have then been true in Ringer's solution also. Evidently the carbon dioxide capacity has been varied by ionic interchanges which are irreversible in the respirometer because there is no outside solution. Had we waited longer for the completion of phos-

phocreatine changes before acidification the differences observed in Ringer muscles would have been slightly greater and the differences in blood muscles slightly less but the sense of the results would have been unchanged. Actually there was very little difference between the rates of volume change in the respirometers at the time of acidification.

On the average, previous treatment with 20 per cent carbon dioxide in blood caused an increase of 9 volumes per cent in the carbon dioxide capacity. This equals 0.4 m.-eq. and may be compared with the average excess potassium found under these conditions in table 4 of 0.5 m.-eq. per 100 grams of muscle. Likewise previous treatment with 20 per cent carbon dioxide in Ringer's solution decreased the carbon dioxide capacity on the average 2.9 volumes per cent or 0.13 m.-eq. which may be compared with the average deficiency of potassium of 0.35 m.-eq. under similar conditions from table 4. This comparison is not quantitatively exact but

TABLE 5

The effect of carbon dioxide on the alkali reserve of muscle

(Cc. of CO₂ combined in 100 grams of muscle equilibrated with 5 per cent CO₂)

SOLUTION	AFTER 5 HOURS IN 0.5 PER CENT CO ₂	AFTER 5 HOURS IN 20 PER CENT CO ₂
Frog blood	18.4	26.4
Frog blood	17.9	29.9
Frog blood	20.2	27.0
Average change during equilibration.....	-6.6	-0.8
Ringer-phosphate	8.1	7.0
Ringer-phosphate	11.1	6.7
Ringer-phosphate	8.2	4.9
Average change during equilibration.....	-3.5	+0.3

the agreement is close enough to suggest that the changes in potassium content at least during immersion in blood are due chiefly to exchanges between K⁺ and H⁺ or to diffusion of potassium as KOH. It is unnecessary to search therefore for an accompanying anion to explain the movement of potassium between muscle and blood.

The potassium content of muscles after 5 hours in Ringer's solution at low carbon dioxide tensions is roughly 7.31 m.-eq. per cent in table 4 (average of 3 only). This is 0.49 m.-eq. per cent less than the corresponding value in blood. Values in table 5 show that under these conditions the muscles in Ringer's solution have a carbon dioxide combining capacity of 9.1 volumes per cent as compared to 18.8 volumes per cent in blood. This is a difference of 9.7 volumes per cent or 0.43 m.-eq. per cent where the potassium loss is 0.49 m.-eq. per cent. While this comparison is not very accurate it suggests that in this case the potassium loss in Ringer's solution

is also chiefly due to diffusion of potassium as KOH to equalize the pH difference between the inside and the outside of the fibres. That this is not always true, however, was shown repeatedly in our previous study of muscles in Ringer's solution of varying pH (Fenn and Cobb, 1934).

Potassium intake from blood of high potassium content. It has been shown that frog muscles immersed in alkaline Ringer's solution with increased potassium content (Fenn and Cobb, 1934) will gain potassium. This is strong evidence in favor of a true membrane equilibrium between muscle and plasma with a membrane permeable to potassium ions, for in order to gain potassium this ion must move against the concentration gradient. On account of the importance of this finding and the relatively few cases in which we have observed it, we performed three experiments in which dry potassium chloride was added to blood in which sartorius muscles were immersed. The amount added was sufficient to increase the normal potassium content three-fold, i.e., from 0.4 to 1.22 m.-eq. per cent. In every case the potassium content of the muscles was increased after immersion as compared to the control muscle analyzed immediately after dissection. The amounts of potassium gained in this way in the three experiments were respectively 0.92, 1.33 and 0.92 m.-eq. per 100 grams of muscle. The change in weight of the muscles was negligible and the amount gained was greater than that needed to raise the concentration of potassium in the tissue spaces to the higher level or even to raise the concentration of potassium in all the muscle water to that level. The potassium was therefore taken in against the concentration gradient.

The intake of potassium as a result of increased concentration of potassium in Ringer's solution surrounding the muscle was found in our former paper (1934) to cause an increase in the bicarbonate content, indicating that some of it went in as KOH; but only about 9 per cent of the potassium diffusion could be accounted for in this way. Two similar experiments were performed in blood. Matched sartorius muscles were immersed, one in normal blood and the other in blood with the addition of approximately 1 m.-eq. of potassium per 100 grams of plasma. Both were equilibrated with 5 per cent carbon dioxide for 5 hours, and then analyzed for bicarbonate (in respirometers). The contents of the respirometers were later rinsed into crucibles and analyzed for potassium. The high-potassium muscles contained 1.35 and 1.05 m.-eq. per cent more potassium than the control muscle and only 0.18 and 0.21 m.-eq. per cent more bicarbonate respectively. This increased carbon dioxide capacity as a result of high potassium could of course be explained by phosphocreatine breakdown which is known to occur (Hegnauer, Fenn and Cobb, 1934) or it may be that all the potassium goes in as KOH most of which, however, is neutralized by lactic acid formation in excess of the phosphocreatine breakdown. In any event these experiments in blood agree closely with our former experiments in Ringer.

Absence of a chloride shift to muscle. Having obtained evidence for a potassium shift we next proceeded to look for a chloride shift from plasma to muscles. For this purpose we proceeded in the same way but analyzed the muscles for chloride content by the Westfall micro method. Other thigh muscles were removed from the same animal at dissection and analyzed for initial chloride by the Van Slyke method. The blood was also

TABLE 6

Chloride gain by muscles in 1 per cent and 9.3 per cent carbon dioxide in blood

PLASMA CHLORIDE		MUSCLE CHLORIDE			CHLORIDE SPACE			Δ WEIGHT	
1 per cent CO ₂	9.3 per cent CO ₂	Initial	After soaking		Initial	After soaking		1 per cent	9.3 per cent
			1 per cent CO ₂	9.3 per cent CO ₂		1 per cent CO ₂	9.3 per cent CO ₂		
m.-eq. %	m.-eq. %	m.-eq. %	m.-eq. %†	m.-eq. %†	%	%	%	%	%
6.75	6.26	0.49	1.60	2.69	7.5	23.7	43.0	-8.6	-9.2
		0.68	1.63	1.45	9.5	24.2	23.2	+2.5	+0.8
		0.75	1.62	1.91	11.5	24.0	30.5	-3.6	-4.3
6.93	6.57	0.71	1.00	1.76	10.5	14.4	26.8	-9.5	-5.3
		0.86	1.06	1.46	12.7	15.3	22.2	-4.6	-4.8
7.19	7.19*	0.78	1.69	1.16	10.9	23.5	16.1	+4.3	+11.5
7.72	7.72	0.83	1.79	1.06	10.7	23.2	13.7	+0.9	-5.5
(8.11)	(5.96)	1.30	2.51	2.07	16.0	30.9	25.5	-5.1	-6.5
7.72	7.63	0.95	2.46	2.44	12.4	31.9	32.0	-1.8	-0.9
Average									
7.26	7.07	0.82	1.71	1.78	11.3	23.5	25.9	-2.8	-2.7

The 1 per cent CO₂ varied from 0.9 to 1.15 per cent in different experiments. The plasma was analyzed after the muscles were removed. The average of the 1 per cent and 9.3 per cent values was used in calculating the initial chloride space. Figures for plasma chloride in parentheses are not included in the average. The 1 per cent CO₂ value for plasma chloride was used in calculations.

* 23 per cent CO₂ instead of 9.3 per cent.

† Per 100 grams weight after soaking.

centrifuged immediately after the muscles were removed and the plasma was analyzed in 0.217 cc. samples for chloride by Patterson's modification of the open Carius method. The results are included in table 6. The averages of nine experiments show that the chloride content after soaking in 1 per cent carbon dioxide was 1.71 m.-eq. per cent as compared to 1.78 in 9.3 per cent carbon dioxide. This close agreement gives a false idea of the uniformity of the experiments, however, for in approximately half

the cases the chloride content was greater in the low carbon dioxide tension while the reverse was the case in the other half, and the differences were rather large.

The last three columns of table 6 show the magnitude of the chloride spaces, or in other words, that fraction of the muscle which must be assumed to contain chloride in the same concentration as that in the external solution. It is calculated by dividing the chloride content of the muscle by that of the solution (cf. Fenn, Cobb, and Marsh, 1934). The averages show that the initial chloride space is only 11.3 per cent of the muscle whereas after soaking it is 23.5 and 25.9 per cent in the 1 per cent and 9.3 per cent carbon dioxide tensions, respectively. There is, therefore, even in the frog's own blood equilibrated with carbon dioxide a large increase in the chloride space, and the chloride content practically doubles. This gain in chloride appears to be independent of carbon dioxide tension as far as can be ascertained by this method. The variations, however, are rather large so that the results do not entirely exclude a possible small chloride shift, which is entirely overbalanced by other more important factors.

We have been unable to discover or control the factor responsible for this chloride gain in blood. Chilling the frog previous to dissection to reduce the stimulation resulting from crushing the brain had no effect. Doubling the amount of heparin causes only a slight increase in the chloride gained which might have been experimental error. Injuring the muscle during dissection by laying it on the frog skin so as to cause a contracture produced likewise only a small increase in the chloride content which was no larger than the variations observed in table 6.

It may be observed also in table 6 that the chloride content of the plasma is slightly less at the high carbon dioxide tension. This can be explained by the well known chloride shift to the corpuscles. There is no apparent correlation between the percentage changes in weight and the chloride gains in table 6. On the average the loss in weight was equal in both high and low carbon dioxide tensions but this varied markedly from one muscle to the next, some gaining weight and some losing weight.

A similar absence of a clean-cut chloride shift was obtained in three earlier experiments in which variations of pH of the solutions were obtained by phosphate buffers, the solutions being equilibrated with pure oxygen rather than with $\text{CO}_2\text{-O}_2$ mixtures. Two of these experiments showed more chloride in the muscle soaked in the more alkaline solution (3.88 and 3.38 m.-eq. per cent in the alkaline muscle compared to 3.78 and 3.29, respectively, in the acid muscles) while the third experiment involving more muscles showed the reverse (3.35 m.-eq. per cent in the acid and 2.76 in the alkaline muscles).

Christy (1927) has described a chloride shift to the tissues in exercise,

and Essen, Kanders and Porges (1932) have described cases in which chloride varies inversely as the alveolar carbon dioxide tension (and the blood HCO_3). These observations, however, are to be explained probably as shifts of NaCl and not as true "chloride shift" where chloride exchanges with another anion. Thus, in exercise Ewig and Wiener (1928) as well as Dill, Talbot and Edwards (1930) have concluded that both sodium and chloride enter the tissues from the blood with a corresponding amount of water.

DISCUSSION. While these experiments show that a potassium shift can occur between plasma and muscle as a result of increased carbon dioxide tension they do not prove that such a shift will actually occur in the human body. It is quite possible that the pH changes which are produced by a rise in carbon dioxide tension will be neutralized by shifts of undissociated lactic acid, a decrease in the lactic acid level in the muscle, or an increased breakdown of phosphocreatine before any potassium can shift. Absence of such a potassium shift in man has actually been reported in experiments by Gollwitzer-Meier (1925).

The chief importance of these experiments is the further evidence which they afford in favor of the conception that a muscle is a potassium-permeable but sodium-impermeable and anion-impermeable structure. Contrary to this view it is now frequently assumed (Peters and Van Slyke, (1931) for example) in the absence of evidence to the contrary, that the tissue cells are comparable in their permeability properties to the red blood corpuscle. All the known facts are equally well explained, so far as we can ascertain, on the theory that potassium is immobilized in the tissue because it is combined with indiffusible anions.

SUMMARY

Frog sartorius muscles, during immersion in frog blood *in vitro*, usually lose some potassium. If the control muscles are immersed a short time in blood to permit loss of potassium from injured fibres and if the blood is equilibrated with a high carbon dioxide tension then the immersed muscles usually gain potassium during immersion. If two matched sartorius muscles are immersed in frog blood one at a high and the other at a low carbon dioxide tension, the muscle in the higher carbon dioxide tension shows the higher potassium content. Measurements of the carbon dioxide combining capacity show that where the potassium content is high the bicarbonate content is high also by an approximately equivalent amount. Muscles immersed in frog blood containing an increased amount of potassium and equilibrated with 5 per cent carbon dioxide show a gain in potassium representing a diffusion of potassium into the muscle against a concentration gradient. No evidence for a chloride shift to the muscle could be found. In frog blood isolated sartorius muscles gain consid-

erably in chloride content and their extracellular spaces are increased in volume, although this change is less in blood than in Ringer's solution.

It is concluded that an increase in carbon dioxide tension tends to cause potassium to shift from plasma to muscles but the same increase in carbon dioxide would cause the potassium to move from the muscles to Ringer's solution because the muscle is intermediate between Ringer's solution and blood in its buffering capacity.

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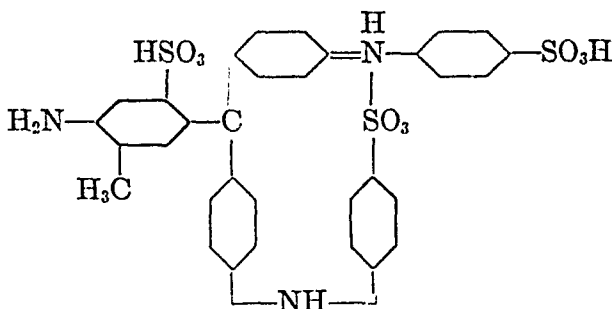
THE KINETICS OF THE ELIMINATION OF THE DYE WATER BLUE FROM DOG PLASMA AFTER INTRAVENOUS INJECTION

A. HEMINGWAY, F. H. SCOTT AND H. N. WRIGHT

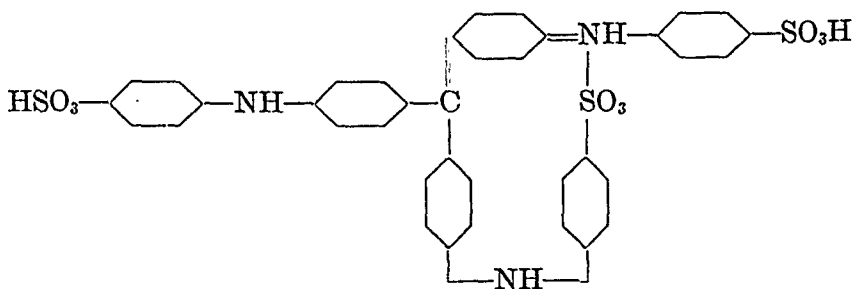
*From the Laboratory of Physiological Chemistry, the Department of Physiology, and the
Department of Pharmacology, University of Minnesota*

Received for publication February 5, 1935

Water blue is a colloidal acid dye formed by sulphonation of the insoluble aniline blue. The dye is probably a mixture containing the di- and trisulphonates of diphenylrosaniline and triphenyl pararosaniline. Two possible components according to Conn (1) are (I) the trisulphonate of triphenyl pararosaniline and (II) the trisulphonate of diphenyl rosaniline, the respective formulas being as follows:



I



II

This dye is also called cotton blue, China blue and water soluble aniline blue. Its physiological properties are its relative non-toxicity in concentrations sufficient to cause intense coloration of living tissues in man (2)

and dogs (3), and its ability to remain in plasma after intravenous injection longer than other known dyes (4). Except for a single excretion curve of Wittgenstein and Krebs (4) and two observations of Dawson, Evans and Whipple (3) made in the hour following injection, there are no quantitative excretory data on this dye.

In order to measure relative plasma volume a dye must be used which is not eliminated to any appreciable extent from the blood stream in the interval during which changes in blood volume are measured. Due to the slow elimination of water blue from the plasma, this dye seemed to fulfill the necessary requirements. In order to test whether or not this dye is suitable for measurements of relative plasma volume accurate excretion data must be known. In the present study elimination rates of the dye from plasma of dogs after intravenous injection have been measured over twelve day intervals. In addition the elimination rate has been measured during a four hour interval several days after the injection. As will be mentioned later these excretion data are confirmatory of the theories of Smith (5) (6), in regard to the removal of dye from plasma by phagocytosis.

METHOD. Water blue (Kahlbaum 3B) was dissolved in sterile saline and injected intravenously into normal unanesthetized healthy adult dogs varying in weight from 10 to 25 kilos. The amount injected was 40 mgm. per milliliter per kilo. Within 3 to 6 minutes after injection the first sample of blood, about 8 ml., was withdrawn from the femoral artery or jugular vein and discharged into a calibrated 15 ml. centrifuge tube containing 1 ml. of 1.6 per cent sodium oxalate. After centrifuging, the cell volume and diluted plasma volume were read. A sample of plasma was removed and mixed with an equal volume of 0.2 N HCl, this developing the blue color to maximum intensity. This first sample of plasma was used as a standard and its relative concentration designated as 100.

Blood samples were obtained at various time intervals after the injection, usually three in the first hour after injection and three more during the next 24 hours, then one sample per day for the following eleven days. In computing relative concentrations of the dye in plasma it is, of course, necessary to consider the hematocrit readings. A simple calculation will show that the relative concentration of the second sample of plasma, C_2 , in terms of the concentration of the first sample, $C_1 = 100$, is

$$C_2 = 100 \frac{1_1 P_1(P_{2+1})}{1_2 P_2(P_{1+1})}$$

where 1_1 and 1_2 are the readings of the Duboscq colorimeter of samples (1) and (2). P_1 and P_2 are the corresponding diluted plasma volumes (diluted with oxalate) of samples (1) and (2). A similar relation holds for subsequent samples.

Turbidity and phenol extraction. In order to develop the intense blue

color acid must be added to plasma. In some cases, usually very few, a turbidity develops on addition of the acid. The turbidity is quite irregular and increases on standing and is probably due to denatured protein. After numerous experiments with HCl and acetic acid of various strength it was found that 0.2 N HCl added in an amount equal to that of the plasma produced maximum color intensity with least turbidity. Where turbidity is produced it can be eliminated by either of the two following procedures.

(1) The use of color filters with a Duboseq colorimeter or the use of a spectrophotometer. The following absorption curve, figure 1, shows the

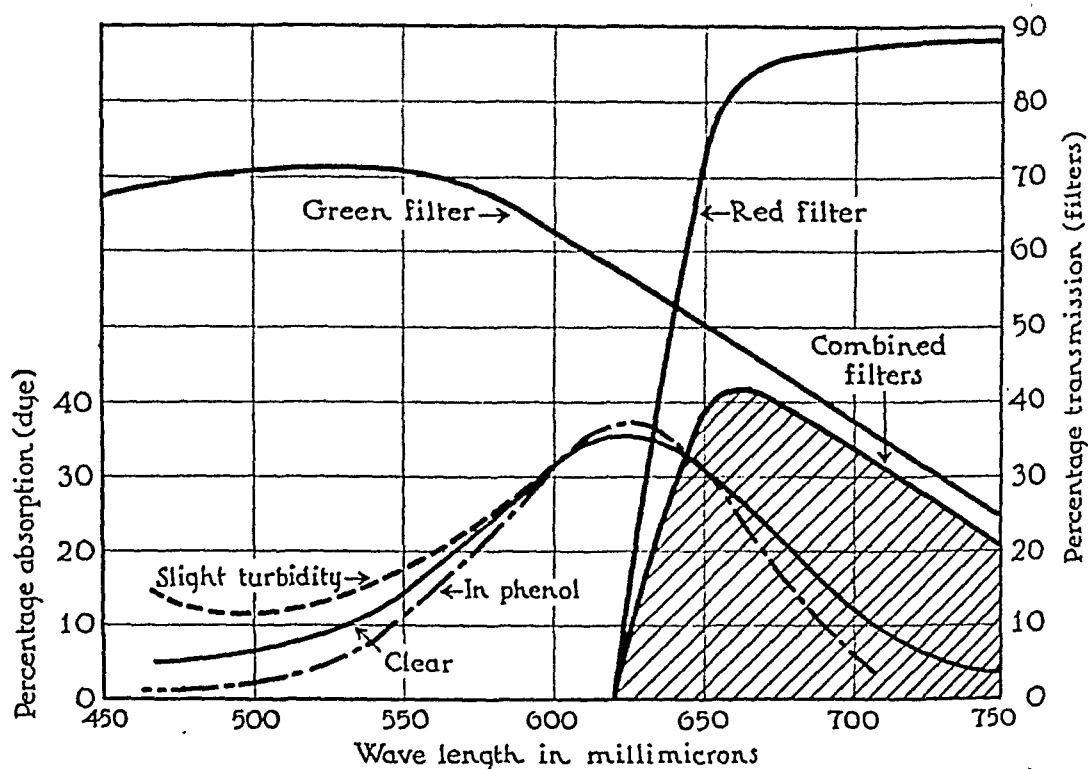


Fig. 1

effect of turbidity on the light absorption of the acidulated plasma containing water blue. Two samples of dyed plasma were chosen, having absorption maxima of approximately the same value, one solution being clear and the other slightly turbid. The effect of turbidity is to increase the light absorption in the blue-violet region of the spectrum. This causes a change in shade of the color, the turbid solution having a reddish tinge, which makes matching in the colorimeter inaccurate. It is to be observed, however, that absorption due to turbidity is much less in the red region of the spectrum. The turbidity error can, therefore, be reduced to an almost negligible amount by using color filters over the eyepiece of the colorimeter,

which permit red light to be filtered through. A suitable light filter combination consists of the Corning filters "H. R." red 3.95 mm. and "heat absorbing" green 2.95 mm. in thickness. The transmitted light lies in a wave length region as given in figure 1.

If the spectrophotometer is to be used rather than the Duboscq colorimeter the prism is set to transmit light having a wave-length $\lambda = 620 \text{ m}\mu$. The turbid solution acidulated plasma is divided into two equal parts, and to one part is added 1 drop of concentrated Na_2SO_3 . This decolorizes the water blue but the turbidity remains. This decolorized sample is then placed in one beam of the spectrophotometer and the colored sample in the other. Measured in this way the turbidity is compensated and the observed absorption is due entirely to the dye.

(2) Attempts were made to precipitate protein with colorless reagents. In all cases where the proteins were completely precipitated the dye was also precipitated. On a suggestion from Mr. H. P. Lundgren it was found that phenol would completely extract the dye from plasma forming a clear non-turbid phenol phase although the plasma itself showed considerable turbidity. For turbid solutions the procedure finally used was to add 5 cc. of phenol saturated with water to the acidulated turbid plasma, shake and centrifuge, jacketing the centrifuge tubes with ice water. After ten minutes' centrifugation the phenol would be a few degrees below room temperature and would contain *all* of the dye. The phenol phase was immediately removed. If the centrifuge tube is not cooled during centrifuging, its temperature will rise a few degrees. On removing the phenol phase and allowing the temperature to drop to room temperature small droplets of water separate, causing a turbidity due to water in the phenol solution. This is prevented by cooling during centrifuging. The error due to cooling the phenol a few degrees below room temperature when separating the two phases, insofar as the phase composition is concerned, is seen to be negligible from the phenol-water solubility curves. Furthermore, all measurements are comparative and are made under identical conditions; hence a slight phase volume change of the phenol phase due to cooling is the same for each experiment.

EXPERIMENTAL RESULTS. I. *The elimination over a twelve day interval.* Figure 2 gives in the form of a graph the relative dye concentration in the plasma of six dogs at various time intervals after intravenous injection. In five cases Kahlbaum's dye "Wasser Blau 3 B" was used. In one case Merck's water soluble Aniline Blue 2 B was used as indicated. It is to be noted that there is a rapid drop in the first few hours after injection followed by a slower elimination lasting 3 to 4 days, then an excretion for the next 7 or 8 days at an approximately linear rate. The resemblance of this excretion data to that of Smith (6) is striking. The general form of the excretion curve of Wasser Blau is similar to that of brilliant vital red (Smith, 6),

differing only in that Wasser Blau after the rapid initial fall disappears more slowly.

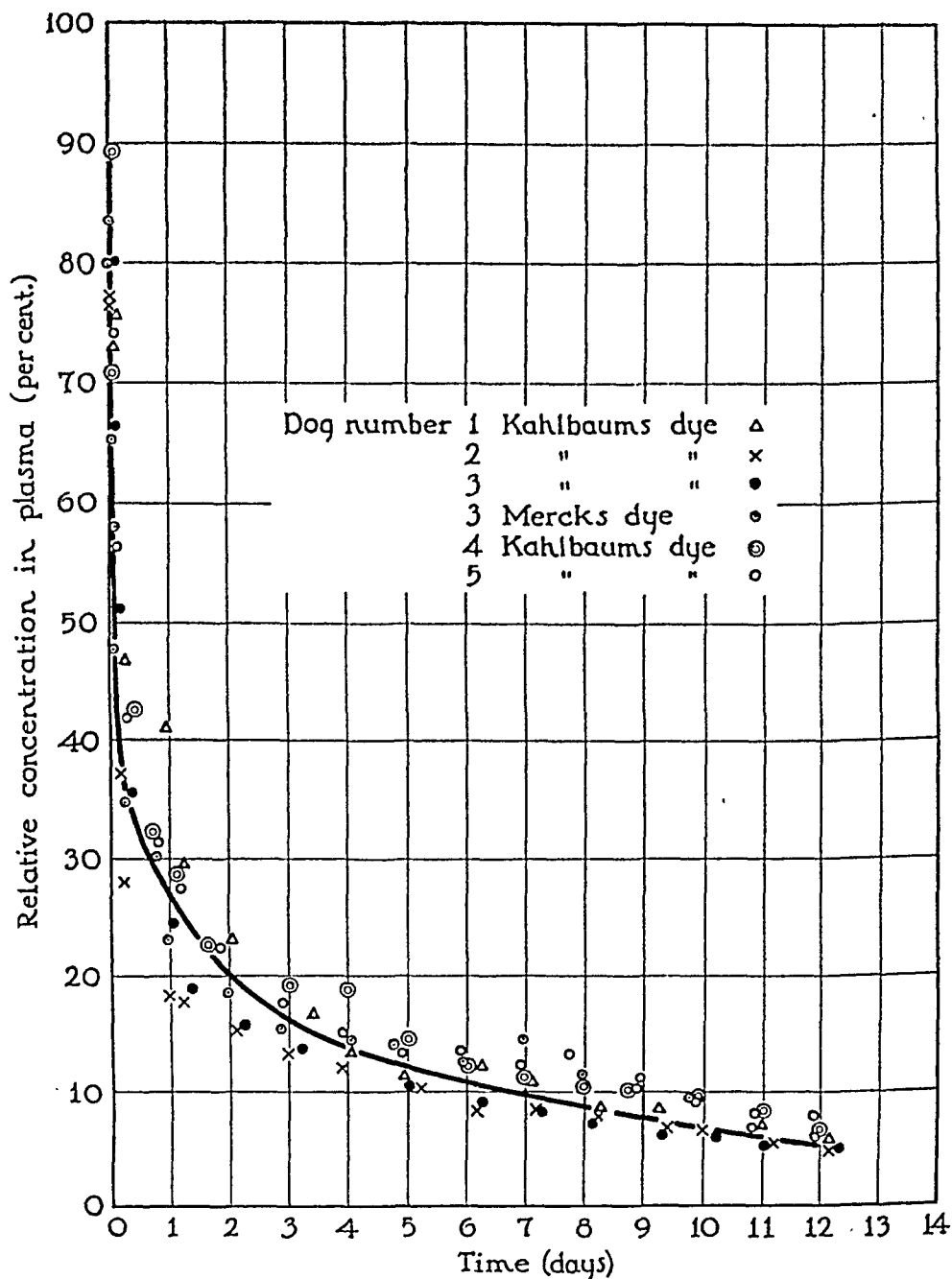


Fig. 2

Smith (5), (6) believes that the dye immediately after injection is removed and stored in the reticulo-endothelial system. The removal is

rapid at first but gradually approaches an equilibrium value where the dye of the cells is in equilibrium with that of the plasma. Superimposed upon this removal into the cells is a slower elimination from the plasma, mainly through the bile. If it be assumed that the plasma dye leaves the plasma to reach finally an equilibrium distribution between cells (reticulo-endothelial) and plasma, then the usual laws of diffusion into a second phase tending to a partition equilibrium may be used. The differentiation equation is

$$\frac{dC_1}{dt} = k(nC_1 - C_2)$$

where k = diffusion coefficient, C_1 is plasma concentration, C_2 is cell concentration and n is the equilibrium partition distribution coefficient $n \frac{C_1}{C_2}$ at equilibrium. A solution of this equation gives the usual exponential relation

$$C_t = C_0 e^{-mt} + D$$

where C_0 is the initial concentration of the dye in the plasma and C_t is the concentration at any time t . If $\ln C_t$ is plotted against t on semi-logarithmic paper a straight line should result. When this plot is made it is found that a straight line does result for the excretion during the first two hours, but after this there is a sudden change of slope and another straight line with a different slope represents the excretion for the next 48 to 72 hours. After this the rate of elimination is almost constant. The excretion can therefore be well represented by the equation

$$C_t = A e^{-at} + B e^{-bt} - vt + D$$

where C_t is the relative plasma concentration of the dye at any time t and A , B , a , b , v and D are constants for each curve independent of C and t . This indicates that there are two processes whereby the dye diffuses from the plasma to a partition equilibrium, namely, a rapid diffusion which is complete in two hours, constants A and a , and a diffusion process which takes place more slowly, constants B and b , this latter process reaching equilibrium after 2 to 3 days. Superimposed on this there is continuous linear elimination at a constant rate v and not proportional to concentration during the time limits of the experiments. A constant elimination independent of concentration shows that this last process is not a simple diffusion. It may be due to activities of the liver cells causing removal of the dye by way of the bile, as suggested by Smith, who observed that such a slow removal of brilliant vital red was not dependent on concentration. In table 1 there is given the curve constants determined for the six dogs

(with averages for the five dogs) in which the Kahlbaum dye was used. From the average values of the constants the continuous curve of figure 2 was drawn, showing a good agreement between the experimental values and the theoretical curve. This agreement lends weight to the excretion theories of Smith (6), his postulates being contained in the above mathematical equation.

TABLE 1

Constants of the curve

$$C_t = A_0 e^{-at} + B_0 e^{-bt} - vt + D$$

where C_t is the dye concentration in plasma at a time t after intravenous injection of the dye.

DOG NUMBER	WEIGHT	V UNITS/DAY	D UNITS	A ₀ UNITS	a (MIN.) ⁻¹	B ₀ UNITS	b (MIN.) ⁻¹
	<i>kgm.</i>						
1	21.3	0.83	16.0	47	9.5×10^{-3}	37	5.3×10^{-4}
2	19.0	0.66	12.5	77.5	7.4	10	2.6×10^{-4}
3*	13.6	1.05	19.0	51	12.5	30	4.0×10^{-4}
3		0.80	14.2	61.5	9.9×10^{-3}	24	5.3×10^{-4}
4	18.4	0.96	18.5	57.5	12.2×10^{-3}	24	3.9×10^{-4}
5	20.5	0.86	17.5	56.5	9.5×10^{-3}	26	3.8×10^{-4}
Average.....		0.82	15.7	60	9.7×10^{-3}	24	4.2×10^{-4}

* Merck's dye used.

TABLE 2

Relative hemoglobin and dye concentration in plasma of normal unanesthetized dogs 6 to 7 days after intravenous injection of 40 mgm. water blue per kilo

DOG NUMBER	DAYS AFTER INJECTION	TIME ELAPSED (HOURS)									
		0		1		2		3		4	
		Dye	Hb	Dye	Hb	Dye	Hb	Dye	Hb	Dye	Hb
1	7	100	100	96.5	90.9	95.0	92.9	87.5	81.1	85.4	80.5
2	6	100	100	96.2	93.2	92.5	89.9	93.4	92.0	89.0	86.3
4	7	100	100	97.1	95.0	91.7	95.0	89.6	95.0	90.9	93.8

II. *Dye excretion over a four-hour interval several days after intravenous injection.* In order to measure more accurately the slow elimination several days after injection a four-hour interval was chosen with blood samples drawn from the femoral artery every hour. The results are given in table 2. In every case the Kahlbaum 3 B dye was used.

From the average excretion curve drawn from the averages of the constants, seven days after injection the relative concentration would be 10.

The average rate of excretion per day is $v = 0.82$ unit per day. During a four hour interval there would be a theoretical drop in concentration due to this slow elimination mechanism from 10 to 9.833, i.e., a drop in concentration in per cent from 100 to 98.3. Actually the measured decrease was much greater than this, amounting to a relative decrease of 10 to 15 per cent instead of the theoretical 2 per cent. Over a four hour interval changes in plasma volume would also cause a change in dye concentration. Relative hemoglobin determinations were made at the same time that the dye concentration was determined. In every case, there was a decrease in hemoglobin for the second, third and fourth, etc., samples; and the decrease in hemoglobin paralleled within the limits of error the decrease in dye concentration. A similar result is to be noted in experiments of Chanutin, Smith and Mendel (7). In a series of hourly samples of hemoglobin drawn from normal unanesthetized dogs there was in 30 cases out of 31 always a decrease (or no change) in the hemoglobin concentration. These results together with ours indicate that successive samples drawn an hour apart give hemoglobin values always lower than the initial sample. The explanation of this decrease in hemoglobin is apparently unknown. It must be taken into consideration in studying changes in the chemical composition of blood over an experimental period when normal unanesthetized animals are used. This hemoglobin change together with the change in dye concentration indicates a dilution of the plasma.

SUMMARY

The elimination of the triphenyl methane dye water blue from dog plasma after intravenous injection has been studied over a 12 day interval. The decrease in concentration is rapid during the first two hours and follows an exponential curve. During the next 48 to 72 hours the elimination follows a slower exponential curve and during the last 7 to 8 days the dye is removed at an approximate constant rate. The concentration plotted against time fits very closely the curve

$$C = A_e^{-at} + B_e^{-bt} - vt + D$$

where C is the concentration at the time t and A , a , B , b , v and D are constants obtainable from the curve and derived theoretically from the theory of Smith (5, 6) in regard to dye removal from plasma.

Six to seven days after the injection of water blue (40 mgm. per kilo per cc. sterile saline) the dye is eliminated slowly at a rate of about 2 per cent over a four hour interval. Repeated sampling at hourly intervals causes an apparent plasma dilution in normal unanesthetized dogs whereby the hemoglobin concentration and dye concentration decrease, the decrease in dye paralleling the decrease in hemoglobin.

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TIME FOR DEVELOPMENT OF INCAPACITY TO WORK IN ADRENALECTOMIZED RATS

W. M. HALES, G. M. HASLERUD AND D. J. INGLE

From The Psychological Laboratories, University of Minnesota

Received for publication February 5, 1935

The demonstration (2) that the intact gastrocnemius muscle of normal anesthetized rats can sustain a high rate of work output for periods of ten days and longer provides an indicator for studying the development of adrenal insufficiency after removal of the adrenal bodies. It was proposed to begin stimulating the muscle of rats immediately following adrenalectomy and to observe the time for development of "fatigue." A preliminary study (3) of this and other problems has been published. The present experiments involve some changes in method.

METHOD. The technique for preparing an animal and the apparatus for stimulating the muscle and recording work have been described (2) in detail. The rats were first selected for resistance to anesthesia by the subcutaneous injection of a standard dose of sodium luminal. The 90 per cent which survived the test were allowed a minimum of two weeks for recovery. For the experimental anesthetization those to be operated received 70 per cent as much luminal as the unoperated controls with ether administered to obtain surgical anesthesia during the operation.

Both adrenals were removed in a single stage aseptic operation by the usual dorso-lateral approach with complete avoidance of postoperative hemorrhage and infection. Six animals were worked simultaneously, the gastrocnemius of each being connected in series and stimulated to lift a 100 gram weight three times per second. Stimulation began within one hour after operation and continued until the death of the animal or to a maximum of 120 hours. At eight hour intervals the experimenter recorded the minute rate and total revolutions from Veeder counters attached to automatic work adders, administered subcutaneously 0.5 cc. distilled water per 100 grams body weight, and injected sufficient luminal to keep the animal immobilized and unresponsive to the faradic shock. Temperature and humidity were constant.

RESULTS. 1. Table 1 summarizes the performance of bilaterally adrenalectomized rats, controls with a kidney and the adrenal of the opposite side removed, and normal animals. The significant comparisons include the rate of work per minute at the end of each eight hour period

TABLE 1

Work records of adrenalectomized, control operated, and normal male rats

GROUP	AGE	WEIGHT	RATE† PER MINUTE AT 8 HOUR INTERVALS										HOURS OF WORK	TOTAL REVOLU- TIONS	
			0	8	16	24	32	40	48	56	64	72			
	<i>days</i>	<i>grams</i>													
I	{ n*	94	214	27	28	26	19	17	17	16	18	18	14	120	132,900
	{ k**	94	214	25	25	25	22	21	22	20	19	19	14	120	137,080
	{ b†	94	214	26	23	11	—	—	—	—	—	—	—	19	20,890
II	{ n	60	225	32	26	21	20	21	19	20	21	22	22	120	157,440
	{ k	60	220	22	27	25	8	7	4	1	1	1	2	120	45,912
	{ b	60	239	26	24	20	3	—	—	—	—	—	—	27	26,040
III	{ n	480	312	38	31	22	12	15	21	25	27	28	22	120	168,060
	{ k	480	316	38	29	22	14	16	20	23	24	23	21	120	156,590
	{ b	480	290	34	16	—	—	—	—	—	—	—	—	10	14,390
	{ b	480	283	27	23	—	—	—	—	—	—	—	—	10	14,940
IV	{ n	480	370	36	37	30	23	23	21	22	30	30	28	120	196,410
	{ k	480	361	37	46	34	21	22	26	28	30	30	29	120	205,090
	{ b	480	375	34	38	17	5	—	—	—	—	—	—	25	38,990
V	{ n	83	249	29	33	26	23	23	22	20	16	19	120	152,920	
	{ k	92	233	27	26	25	16	13	12	12	10	8	8	120	87,640
	{ b	94	264	38	34	4	—	—	—	—	—	—	—	23	29,620
VI	{ n	62	192	30	30	25	13	+0	+0	+0	2	4	6	120	63,355
	{ k	62	198	26	27	26	24	22	26	23	22	20	19	120	150,410
	{ b	62	201	20	—	—	—	—	—	—	—	—	—	7	4,660
	{ b	62	195	24	—	—	—	—	—	—	—	—	—	6	15,250
VII	{ n	93	200	28	22	21	20	21	19	21	23	22	20	120	125,070
	{ k	93	200	28	23	19	13	14	13	15	15	15	14	120	83,420
	{ b	93	210	29	19	14	10	—	—	—	—	—	—	32	24,790
VIII	{ n	74	195	34	26	28	21	14	15	12	15	3	+0	120	76,610
	{ k	74	204	29	28	24	24	24	22	24	24	23	22	120	159,240
	{ b	74	207	29	25	20	4	—	—	—	—	—	—	28	28,580
IX	{ n	80	197	30	31	27	23	22	21	22	25	26	24	120	170,630
	{ k	80	197	27	23	20	9	4	12	+0	+0	+0	+0	120	33,575
	{ b	80	197	36	29	17	—	—	—	—	—	—	—	23	28,575
X	{ n	?	316	45	42	38	30	32	28	28	30	28	29	120	215,990
	{ k	?	318	42	40	36	28	29	28	28	30	28	30	120	207,740
	{ b	?	322	45	20	7	—	—	—	—	—	—	—	30	12,590

* Normal.

** Left adrenal and right kidney removed.

† Bilaterally adrenalectomized.

‡ Recorder revolutions each approximating 400 gram-centimeters work.

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up to 72 hours, total hours of work, and total work accomplished before "fatigue" or a maximum of 120 hours.

It is apparent that the capacity for sustaining work among animals subjected to severe control operations compares favorably with that of unoperated animals. In contrast the completely adrenalectomized animals work normally for only a short time before developing a breakdown of muscular responsiveness.

2. Among a total of 45 bilaterally adrenalectomized animals which began work immediately after operation 6.6 per cent exhibited breakdown by the 8 hour period, 53 per cent by 16 hours, 78 per cent by 24 hours, 96 per cent by 32 hours, and no animal exceeded 36 hours. When the opposite leg was stimulated immediately after the breakdown, it contracted normally at first but lost its capacity for response within a few minutes. The animals succumbed on the average $2\frac{1}{2}$ hours after cessation of muscular contractions.

3. That anesthesia shortens the survival period of adrenalectomized animals is well known as is also the observation (1) that exercise hastens the manifestation of adrenal insufficiency. To test these two conditions one each of 20 like-sex litter-mate pairs matched for weight was subjected to work of the gastrocnemius muscle, while the control animal was treated in an identical manner except for the omission of the weighting and stimulating of the muscle. The "work" animals survived from 10 to 40 hours with an average of 26 hours, while the "no-work" group ranged from 10 to 211 hours with an average of 64 hours. In only one instance did a "no-work" animal die before its "work" control.

4. Evidence of the demands of continuous muscular contraction and of anesthesia upon the adrenalectomized organism is apparent from comparing results, 1, 2 and 3 with the survival under optimal living cage conditions of 42 bilaterally adrenalectomized rats which lived from 7 to 32 days with an average of 20 days.

5. Administration of adrenal cortex extracts¹ not only effectively prevents the breakdown of anesthetized working adrenalectomized rats but also brings about recovery once "fatigue" has occurred. This problem is the subject of a detailed investigation and will be treated in a later report.

The results of these studies substantiate those of a preliminary report (3).

DISCUSSION. The rat has not been universally accepted as a suitable animal for studies of adrenal insufficiency. The striking lack of uniformity among results of such studies on these animals is probably due to individual or strain differences in amount of accessory cortical tissue, differences in diet, particularly in amount of sodium chloride included, and incomplete removal of the gland. In these experiments diet was constant, and it is

¹ Efficacious extracts from the adrenal cortex were made available to us through the kindness of Dr. E. C. Kendall, The Mayo Foundation and Dr. Oliver Kamm, Parke, Davis & Company.

manifest that accessory tissue, although a likely factor producing individual differences in work and survival has not prevented the demonstration of adrenal insufficiency in the rat either in the optimal living cage situation or under conditions of stress. As to technique the animals were prepared by one of us who had already the experience of performing several thousand adrenalectomies.

It is necessary to examine the possibility that operative shock rather than glandular insufficiency accounts for the breakdown of working adrenalectomized animals. Several considerations, however, point to the absence of the adrenals themselves as the essential factor. Operative shock is difficult to produce in the rat. The removal of one adrenal and the opposite kidney as in the control operations should provide a trauma at least equivalent to that from bilateral adrenalectomy, but the performance of the operated controls is like that of the normals and clearly differentiated from that of the experimentals. Moreover, the breakdown occurs several hours after operation, much later than operative shock would be expected. Finally, the fact that the adrenalectomized animals can be sustained with cortin provides strong evidence that one is concerned with an insufficiency of this hormone.

The loss of capacity to work under the experimental conditions cannot properly be regarded as fatigue although the performance of work hastens the development of the deficit. When the unworked gastrocnemius is stimulated after "fatigue" of the working leg, it contracts normally but "fatigues" within a few minutes. This, together with the observation that body temperature is lowered and that a general cyanosis appears supports the authors' view that the condition is best described as an "adrenal shock" and that the decrement in intensity of contraction reflects the onset of shock.

In addition to work and anesthesia several other factors probably contribute to the final breakdown of the experimental animals. Distilled water was injected subcutaneously, an unphysiological procedure but one well tolerated by normal rats and even adrenalectomized animals in early stages of insufficiency. When work output has dropped to a low level, the injection of water may be followed by the abolishment of muscular contractions within very few minutes, and possible death. The marked edema which develops in the extremities of the working adrenalectomized animal may provide toxic substances. But while certain factors may contribute to the differences observed between the work capacity of normal and adrenalectomized animals, the results unequivocally require absence of the adrenal bodies as the necessary condition.

SUMMARY

Bilaterally adrenalectomized rats in optimal living quarters survived from 7 to 32 days. Similar animals anesthetized with sodium luminal and

receiving water by subcutaneous injection lived from 10 to 211 hours or an average of 64 hours. Stimulating the weighted muscle of animals matched with the previous group for every other experimental condition lowered the average survival period to 26 hours with a range from 10 to 40 hours.

Adrenalectomized, anesthetized animals forced to work immediately after operation performed normally at first but within a maximum of 36 hours developed a "fatigue" which may be regarded as adrenal shock. Operated controls worked like normal animals. The "fatigue" of the adrenalectomized animals may be prevented or reversed by administration of adrenal cortex extracts.

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THE INACTIVATION OF HISTAMINE IN PERFUSED ORGANS

FREDERIC R. STEGGERDA, HIRAM E. ESSEX AND FRANK C. MANN

From the Department of Physiology, University of Illinois and the Division of Experimental Medicine, The Mayo Clinic, Rochester, Minnesota

Received for publication February 23, 1935

A series of investigations in this laboratory has been devoted to the site of inactivation of a number of alkaloids with special reference to the part played by the liver in their elimination from the blood of the dog. Priestley, Markowitz and Mann (1931) demonstrated that the liver was more effective in removing strychnine than other tissues of the body. Biebl, Essex and Mann (1932) found the same to be true in the case of nicotine. The liver did not appear to be more capable of eliminating ephedrine from the blood than the other tissues studied by Thorp, Essex, and Mann (1933).

The inactivation of histamine was the subject of an extensive study by Best and McHenry (1930). The perfusion experiments carried out by these workers were done by means of the Dale-Schuster system of diaphragm pumps.

It was thought that an investigation of the inactivation of histamine in a manner similar to that of previous studies reported from this laboratory might prove of value. It should be stated here that with the exception of the results with the perfused liver, our findings are in agreement with the perfusion experiments of Best and McHenry.

METHODS AND RESULTS. As in previous studies, the isolated organs (hind limbs, liver, intestines, and kidneys) were perfused by the use of the Starling heart-lung preparation. Defibrinated blood, containing a small amount of heparin, was used for the perfusing medium. About 1000 cc. of blood were used in each experiment.

The degree of inactivation of histamine was determined by the effect of successive samples of the perfusing blood on the blood pressure of dogs, weighing from 6 to 8 kgm., which were anesthetized with sodium amytal.

In each experiment the histamine was added to the venous reservoir of the heart-lung preparation, and after thorough stirring a control sample was removed. The doses of histamine employed varied from 20 to 100 mgm. Two experiments were sufficient to indicate the inadvisability of doses as great as 100 mgm. The heart-lung preparation survived doses of this size but maximal effects were obtained on the blood pressure of the test animal when 10 cc. of blood from the heart-lung-liver preparation were

given. It was therefore difficult to determine whether inactivation had occurred even though the blood containing histamine had been perfused through the heart-lung-liver preparation for nearly three hours. Consequently, doses of from 20 to 50 mgm. were used in subsequent experiments.

Heart-lung preparation. It was first necessary to determine the ability of the heart and lungs to inactivate histamine. In two experiments 20 and 30 mgm. of the drug, respectively, were used. After three hours' perfusion such a small amount of the amine had been removed that the use of the heart-lung preparation for perfusing other organs did not appear open to serious objection.

Heart-lung-hind limbs. The hind limbs were prepared for perfusion according to the method employed by Thorp, Essex, and Mann, which briefly described consists of placing the hind limbs of another dog, weighing from 6 to 8 kgm., in the heart-lung circuit by cannulating the abdominal aorta and vena cava in the lower portion of the abdomen, the remainder of the animal being eliminated by appropriate ligatures and sectioning. By this method the limbs were kept in excellent condition and responded to electrical stimulation in a normal manner at the end of the experiment. In such a preparation a dose as small as 25 mgm. was not inactivated after perfusion for one hour and forty-six minutes. There was, however, in three experiments a much greater reduction of histamine than occurred with the heart-lung preparation alone.

Intestine. The intestine was prepared for perfusion as follows: 1, the colon was sectioned anterior to the inferior mesenteric artery; 2, the duodenum was sectioned in the region of the tail of the pancreas, and 3, the mesentery was cut and the intestines were freed except in the region of the superior mesenteric artery and portal vein, which were then cannulated. After this the intestines were placed in a saline bath which was kept heated at body temperature. As soon as possible after removal, the inferior mesenteric artery was placed in the arterial circuit of the heart-lung preparation and perfusion was begun. By this technic the intestines were without arterial blood not longer than two minutes. After the preparation was completed, 25 mgm. of histamine were added to the venous reservoir and the same procedure as was employed in the other experiments was followed. As indicated by the blood pressure of the test animal, all of the histamine had not disappeared after an hour's perfusion. The results of two satisfactory experiments indicated that the perfused intestine does not inactivate histamine more rapidly than the perfused hind limbs.

Liver. The liver was prepared for perfusion by a method that permits an uninterrupted supply of arterial blood in the hepatic artery. After the heart-lung preparation had been made, the liver of another dog weighing from 6 to 8 kgm. was prepared for perfusion. This was accomplished by isolating the hepatic artery, around the proximal portion of which a loose

ligature was placed. The gastroduodenal branch of the hepatic artery was ligated, cannulated, and placed in the circuit of the heart-lung preparation. At the same time the ligature about the hepatic artery was tied and the artery sectioned. The portal vein was cannulated and perfused from the venous reservoir. The common bile duct was ligated, and the liver was removed in the usual manner and placed in a container from which an outlet carried the blood from the liver to the venous reservoir of the heart-lung preparation.

As compared with the hind limbs, the perfused liver eliminated histamine from the blood decidedly more rapidly. In a typical experiment 30 mgm. of the amine were eliminated in one hour and forty-five minutes. In another experiment 20 mgm. of histamine had practically disappeared in forty-five minutes.

Kidneys. Since the findings of Best and McHenry that the kidneys are more effective in eliminating histamine than the other organs perfused, it seemed particularly desirable that optimal conditions accompany the perfusing of these organs. The following method was used, which insures a minimum of disturbance of the kidneys in their preparation. As in the preparations of Best and McHenry, all branches of the aorta were ligated and sectioned, from the level of the superior mesenteric artery to a point posterior to the internal spermatic artery, at which point the aorta and vena cava were cannulated. A loose ligature was placed around the aorta and vena cava just distal to the superior mesenteric artery. The cannula in the aorta was connected with the arterial side of the heart-lung preparation. When this was completed, the loose ligatures about the aorta and vena cava were tied and, simultaneously, the clamps were removed from the aorta and vena cava, thus allowing the kidneys to be perfused at a blood pressure within physiologic limits. By this means the kidneys were never without arterial blood. By leaving the kidneys in situ, the trauma incident to removing them from the body was avoided, thus affording a more nearly normal preparation. The results obtained with such preparations are in striking contrast to those obtained with the other preparations described. Practically all of 25 mgm. of histamine was removed from the blood in three instances within fifteen minutes after the amine was added to the venous reservoir. These experiments amply confirm those of Best and McHenry and furnish additional proof of the ability of the kidneys to eliminate histamine from the blood.

SUMMARY AND CONCLUSIONS

A study has been made of the inactivation of histamine by organs perfused by means of the Starling heart-lung preparation. The degree of inactivation was determined by the effect on the blood pressure of a test animal.

The heart and lungs, hind limbs, small intestine, liver, and kidneys were studied from this point of view. The kidneys were most effective of all the organs studied in eliminating histamine, since they inactivated as much as 25 mgm. in fifteen minutes. The liver was less effective than the kidneys but was more effective than the other tissues studied in inactivating this amine.

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INCREASED SUSCEPTIBILITY TO LOCAL INFECTION FOLLOWING BLOCKAGE OF LYMPH DRAINAGE

CECIL K. DRINKER, MADELEINE E. FIELD, HUGH K. WARD AND
CHAMP LYONS

From the Department of Physiology, Harvard School of Public Health, and the Department of Bacteriology, Harvard Medical School, Boston, Mass.

Received for publication February 7, 1935

Lymphatic obstruction in human beings becomes a crippling disease for two reasons. These are, first, the gradual development of elephantiasis and, second, repeated attacks of acute lymphangitis with local disability and general prostration. Such attacks are not invariable in elephantiasis but when they occur they accelerate the proliferative processes and are in themselves a serious cause of disability.

In recent papers, Drinker, Field and Homans (1934) and Drinker, Field, Heim and Leigh (1934) have described the production in dogs of edema and elephantiasis due to lymphatic obstruction, and have presented detailed examinations of the edema fluid as elephantiasis progresses. In those papers, acute inflammatory attacks were mentioned. These occurred spontaneously in the affected legs of certain of the animals, and could be induced by injection of a hemolytic streptococcus into the edematous part, the organism having been isolated from one of the animals early in a spontaneous attack. It is the purpose of the present paper to describe such attacks in detail since they appear to be one expression of a loss of function ordinarily provided by the lymphatics.

Since filariasis is much the most frequent cause of elephantiasis, it is appropriate to review briefly current conceptions of the etiology of the condition and thus to gain an idea of the fundamental identity of the disease in human beings and the condition which develops in dogs deprived of lymph drainage. Lymphatic obstruction in filariasis depends, first of all, on the presence of female filaria in the lymphatics of the affected part. Through some means, not certainly understood, these organisms induce fibrotic changes leading to obstruction. This is added to by dead ova and dead microfilaria. Lymph stasis results and very frequently attacks of lymphangitis. The part enlarges due in the beginning to edema but very soon to fibrous overgrowth. At the present time there is a controversy as to whether elephantiasis due to filariasis can occur as a result of lymph stasis alone or whether bacterial infection is also essential. O'Connor

(1932) has provided an excellent review of the mixture of knowledge and opinion which has caused this controversy. Our observations must not be thought of as settling it. All they show is that dogs which are normally highly resistant to streptococcic infection become locally very susceptible to these organisms if they are injected into a part edematous from lymphatic obstruction, and that just as in human elephantiasis these dogs, in certain instances, experience spontaneous attacks of lymphangitis. Early in these attacks a hemolytic streptococcus may be cultivated from the edema fluid. The bearing of these findings upon the problems of filariasis is for others to settle. It is, however, possible that our experience may clarify the methods to be used in arriving at more satisfactory clinical results.

The attacks of infection. During the past two and a half years we have produced some measure of lymphedema and elephantiasis in a number of animals. Most of these have been sacrificed relatively early in the disease caused by lymphatic obstruction, in order to obtain information available through a terminal experiment with autopsy. Five dogs have been given marked lymphedema and have been studied over long periods. Of these, 4 have developed elephantiasis in greater or less degree, the degree of overgrowth being determined by the appearance of fistulae. Dogs subjected to lymph blockage experience a tremendous dilatation of lymph capillaries, particularly in the skin. These vessels are prone to rupture through the thinned epithelium, and fistulae result which drain and close and then break out again so that the part is never subjected to true stagnation of edema fluid. When fistulae occur frequently, not only does one observe less elephantiac overgrowth, but local infection, such as we shall describe, is relatively uncommon.

An abridgment of the history of dog 1¹ will provide a characteristic account of our experiences with streptococcic infection in experimental lymphedema and elephantiasis. On May 5, 1933, when permanent swelling of the left hind leg had been established and lymphatics could no longer be found, it was noticed that this dog was unwell. He favored his left hind leg in walking and it was slightly tender. At the time, we failed to recognize what was taking place and within 36 hours the animal was active once more. Ten days later, May 15, the same thing happened again. The attack began at 4:30 p.m. The left leg was swollen, hot, and painful on motion though not so to touch. Three hours later the temperature² was 105.8°F. and the leucocyte count in the blood 25,200 per cubic millimeter. Edema fluid flowed freely on puncture. It had a protein

¹ The course of elephantoid growth and the composition of the edema fluid in this animal have been described. He is dog 1 of the first two papers in the bibliography of this paper. Dog 2 is dog 2 in the same papers.

² All temperatures are rectal.

content of 2.36 per cent, and 58,300 white cells per cubic millimeter of which 90 per cent were polymorphonuclear leucocytes. Next day fever continued, but on the third day was gone and the animal was entirely recovered. Cultures of the edema fluid and blood made upon the second day were sterile.

The third experience occurred upon June 23, slightly over a month later. At this time the dog was living in the country, as one of several house dogs. He was active and except for the appearance of his hind legs, wholly normal. At 4:00 p.m. on the 23rd it was noticed he was limping. An hour later a hard chill began and the temperature was 103.4°F. It rose steadily and at 8:00 p.m. was 106.7°F. At that time leucocyte counts in blood and edema fluid were 15,800 and 37,800 per cubic millimeter, respectively. A hemolytic streptococcus was isolated from the first specimen of edema fluid at 8:00 p.m., but not again during this attack. Blood cultures were consistently sterile. The animal had fever throughout June 24, but by the afternoon of the 25th was again well.

A fourth attack occurred on July 26, again without the slightest warning. On this occasion a hemolytic streptococcus was again found in pure culture in the edema fluid taken in the first 3 hours of the attack, and blood cultures obtained simultaneously were sterile. On the 28th the dog was entirely recovered.

From May 5, 1933 until November 19, 1934, dog 1 has had 14 of these spontaneous attacks in the left hind leg and 3 in the right leg, which is somewhat edematous and elephantiac but which has never been pushed as far as the left. Figure 1 gives the detailed findings in a typical seizure, that of September 5, 1933. When this attack occurred the dog was living in the country. At 5:30 p.m. it was noticed he was limping. Fifteen minutes later he had a severe chill. His temperature at 5:55, as shown in figure 1, was 102.2°F. It rose rapidly and by the time the laboratory was reached was 106.4°F. From this point it fell until a little after 2:00 a.m. when a second rise began which subsided during the afternoon and following night. The edema fluid gave positive cultures of hemolytic streptococci during the first 12 hours of observation. It then became sterile and remained so until a final specimen was taken on the morning of September 7, which turned out to be positive. This and one other occasion are the only times upon which a positive culture has been obtained except early in an attack. Blood cultures were taken with each specimen of edema fluid. All were negative.

The record displayed in figure 1 becomes rather more graphic when compared with figure 2. This chart shows the result of injecting 13 cc. of a dilute culture of hemolytic streptococci isolated from the same dog in a previous spontaneous attack. The animal, dog 1, weighed 41.7 kgm. and the left leg into which the organisms were injected was very large and filled

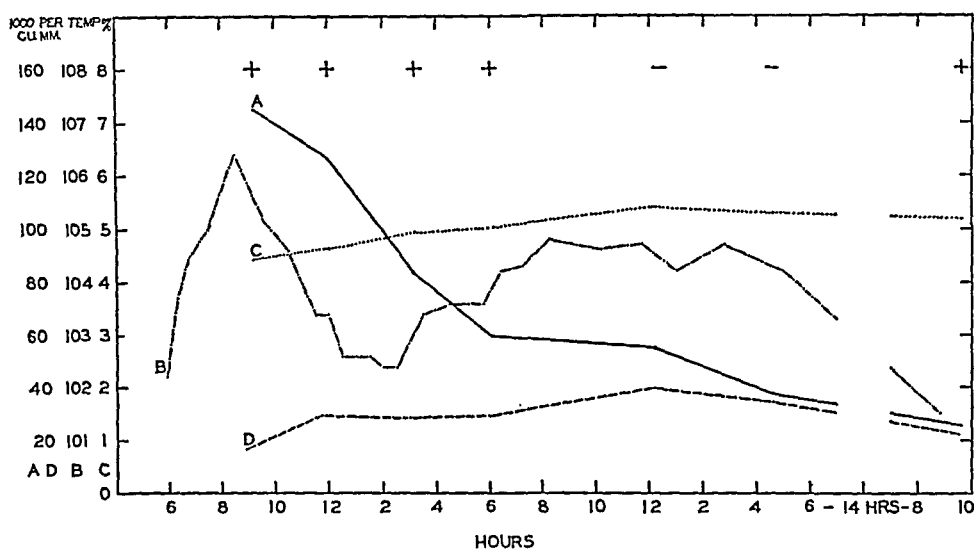


Fig. 1. Details of a typical infectious attack in the left hind leg of dog 1, September 5, 1933. *Line A*, leucocytes in edema fluid in thousands per cubic millimeter; *line B*, rectal temperature in degrees Fahrenheit; *line C*, total protein in edema fluid in grams per cent; *line D*, leucocyte count in blood per cubic millimeter. Ordinates, as given for the different curves; abscissae, in hours. Plus signs indicate cultures of edema fluid positive for hemolytic streptococci; minus signs, negative cultures.

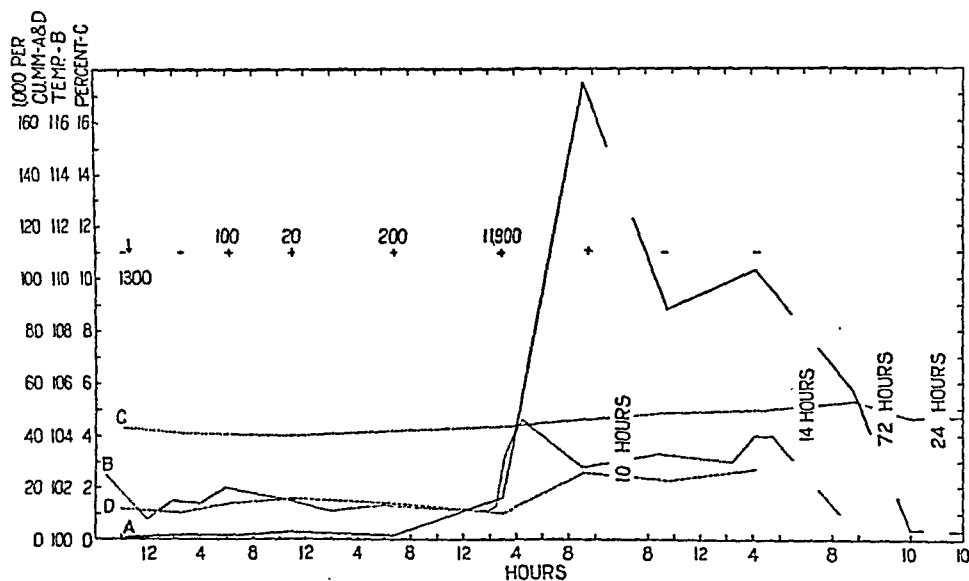


Fig. 2. Details of an infectious attack in dog 1, April 4, 1934, induced by subcutaneous injection of a dilute culture of hemolytic streptococci isolated from this animal in a spontaneous attack. Charting identical with figure 1. At the arrow, 0.3 cc. per kilogram of a dilute culture containing 1300 colonies of streptococci per cubic centimeter was injected in divided amounts under the skin of the left leg. Subsequent figures indicate the number of colonies per cubic centimeter recovered in specimens of edema fluid.

with edema fluid. Control experiments on normal dogs had shown that cultures of this streptococcus containing more than 100,000,000 colonies per cubic centimeter in doses of 0.3 cc. per kilogram were required in order to produce a measurable increase in temperature and blood leucocyte count in normal dogs. In this experiment, 1300 colonies per cubic centimeter were used. We have, however, seen reactions after injection of this streptococcus in concentrations as low as 25 colonies per cubic centimeter, the culture being injected subcutaneously in a lymphedematous leg in an identical amount per kilogram.

Following the injection shown in figure 2, there were no symptoms until 30 hours had passed. The dog then had a chill and a typical attack. There was a tremendous outflow of leucocytes into the edema fluid (curve A, fig. 2) and at the height of this reaction no organisms were recovered by

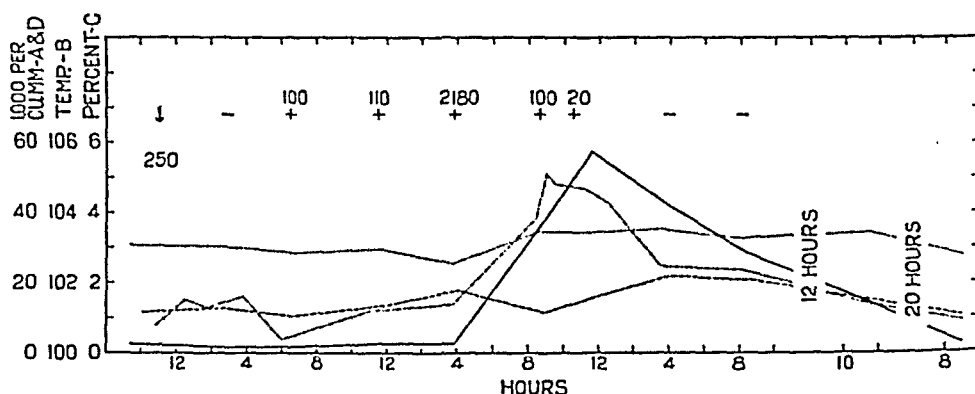


Fig. 3. Details of an infectious attack in dog 2, December 15, 1933, induced by subcutaneous injection of 0.3 cc. per kilogram of a dilute culture containing 250 colonies per cubic centimeter of a hemolytic streptococcus isolated from dog 1 during a spontaneous attack. Charting identical with figures 1 and 2.

direct plating but a few were found later by plating from bouillon tubes. Subsequent cultures were negative. As in spontaneous attacks blood cultures were taken regularly and were sterile.

The experiment shows growth of the organism planted in the edematous leg and a characteristically explosive reaction when, however, the number of colonies in the edema fluid was not great.

Figure 3 is the graphic record of another similar experiment. In this case the same organism was injected into the edematous and elephantiac leg of dog 2. Dosage was the same per kilogram and the number of organisms in the culture used equalled 250 colonies per cubic centimeter. The result is very similar to that seen in figure 2. The organisms increased in the edema fluid for some hours and when a very moderate number was reached, 2180 colonies per cubic centimeter, a pronounced reaction occurred with a heavy outflow of leucocytes in the edema fluid and rather prompt sterility.

The attacks, both spontaneous and induced, have had certain characteristics in common. First of all, blood cultures have never been positive in the transient seizures. Second, the attacks with a single exception have been over in 3 days. Third, a hemolytic streptococcus was the single organism isolated repeatedly from the edema fluid during attacks. Staphylococci and diphtheroid organisms have been obtained occasionally but have had no uniform place in the history of the disease and have been considered to be contaminating organisms. Fourth, except upon two occasions, both in the case of dog 1, no streptococci have been isolated from the edema fluid later than the twelfth hour after the onset of an attack. Fifth, repeated attempts to find hemolytic streptococci in the edema fluid between attacks have failed completely.

The fourth of these "common characteristics" which mentions two exceptions requires comment. It has been shown in figure 1 that in a spontaneous attack experienced by dog 1, a positive culture of hemolytic streptococci was secured 40 hours after the onset of the attack and following two collections of fluid which proved sterile. No reason can be offered for this other than the entrance of the needle into a small pocket of fluid which still contained living organisms. Until very recently the attacks of lymphangitis, though severe, have been brief. This is the ordinary experience in human cases. But on November 23, 1934 a more serious seizure was encountered. Four days previously, dog 1 wandered off with another dog belonging to one of the authors. In the next days he was heard from twice and at an increasing distance from home. He was recovered on the morning of the 23rd and had lost greatly in weight and strength. It was at once noticed that the dog was lame in the right hind leg. His temperature was 103.9°F. Edema fluid from the right leg contained 171,000 cells per cubic millimeter and a hemolytic streptococcus was recovered from it. As the left leg was also hot, fluid was taken from it. This fluid contained 78,000 cells per cubic millimeter and was also positive for hemolytic streptococci.

A very serious illness now began in which cultures of edema fluid from both legs were repeatedly positive. Abscess formation occurred in the right leg. This evacuated spontaneously on November 26. The illness became a series of attacks, both legs being involved. Every three or four days a typical seizure occurred with high fever and great prostration. On December 14, under nembutal anesthesia, multiple incisions were made in both legs. The fluid which escaped was relatively clear and had a protein content of 4.5 per cent. Defibrinated blood from the animal killed the infecting streptococcus, and it was thought that a rapid renewal of the edema fluid might result in a medium less favorable for growth. This supposition proved wrong. The illness continued and finally the afebrile periods disappeared. On January 26 the dog was anesthetized

and killed. Blood cultures at this time gave a rich growth of hemolytic streptococci, and the same organism was obtained from the edema fluid and from tissues taken from all parts of the body.

The infecting streptococci. A hemolytic streptococcus was isolated first from dog 1 on June 23, 1933. An organism identical in cultural characteristics was then isolated repeatedly from this dog and from two other animals in similar condition. This streptococcus was killed by the whole blood of normal dogs and of dogs that had had attacks of lymphangitis. It grew in the edema fluid taken between attacks. It was avirulent for mice, and when inoculated subcutaneously in normal dogs produced no reaction until enormous dosage was reached. Inoculated in tissue with blocked lymphatics a characteristic attack was induced.

Many attempts were made to obtain this organism from edema fluid between attacks. All were failures. Cultures from muscle and lymph nodes were likewise negative.

The series of attacks in dog 1 which began on November 23, 1934 were due to a different hemolytic streptococcus. This organism at first produced isolated attacks and finally a fatal illness. It proved very virulent for mice, contrasting markedly with the first streptococcus. Whereas in the case of the first organism the undiluted culture was non-fatal for mice, as few as twenty of the second streptococcus caused death. Injected subcutaneously in normal dogs it caused local disturbance with fever in relatively small dosage. The reaction was, however, mild when compared with the result of injecting such an avirulent organism as streptococcus 1 into lymphedematous parts.

DISCUSSION. The experiences which have been described bring out, first of all, the extreme susceptibility to streptococcic infection which follows lymphatic obstruction. Not only is infection easily possible but, as in the case of the first streptococcus encountered, the infecting organism may be of low virulence and yet capable of causing severe illness. It has not been possible to learn the origin of the infecting streptococci. Large amounts of culture of the first organism have been injected intravenously with no result. Spontaneous attacks on three occasions followed severe muscular work, but efforts to induce an attack by heavy exercise have not been successful.

The uniformity with which streptococci have been isolated from edema fluid is not in accord with human experience. But here again our procedure has been somewhat different. Edema fluid can be collected from many cases of human elephantiasis if the part is allowed to be dependent for a half hour prior to puncture. In order to get fluid for culture it should not be necessary to inject salt solution and withdraw it. Furthermore, it is imperative that cultures be made as early in the attack as possible. The induced attacks shown in figures 2 and 3 indicate forcibly enough that

the highest concentration of organisms occurs with the very onset of the disturbance.

SUMMARY

1. Dogs subjected to lymphatic blockage develop a local susceptibility to infection by hemolytic streptococci.

2. Attacks of chills and fever with local inflammation occur spontaneously in such animals.

3. A hemolytic streptococcus can be isolated from the edema fluid early in such attacks.

4. Blood cultures have never been positive during characteristic brief attacks.

5. The source of the infecting organism has not been determined. Large dosage through the blood stream has not produced attacks.

6. These results indicate the importance of normal lymphatic drainage in resisting infection.

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THE USE OF HYPERTONIC SUCROSE SOLUTION INTRAVENOUSLY TO REDUCE CEREBROSPINAL FLUID PRESSURE WITHOUT A SECONDARY RISE

L. T. BULLOCK,¹ M. I. GREGERSEN AND R. KINNEY

From the Laboratories of Physiology in the Harvard Medical School

Received for publication January 16, 1935

The methods used for reducing high intracranial pressure fall into three groups: 1, direct relief of pressure by opening the skull; 2, cerebrospinal fluid drainage, usually by lumbar puncture; and 3, dehydration of the brain and ventriculo-subarachnoid system by changing, either directly or indirectly, the composition of the blood. Each of these methods is subject to distinct limitations.

Inasmuch as the cranial contents are not fluid and the cranial cavity is subdivided by more or less rigid structures (tentorium and falx cerebri), operative decompression may fail to reduce the pressure equally throughout the brain and lead to local congestion, sudden swelling and edema, which entirely defeat the purpose of the operation (Heuer, 1929; Munro, 1934). Lumbar puncture, especially when the intracranial pressure is high, is regarded by some as a hazardous procedure (Cushing, 1909; Homans, 1932; Bailey, 1932; Masserman and Schaller, 1933); although Masson (1929), in an analysis of 200 cases, tends to minimize its dangers. The third method, introduced by the experiments of Weed and McKibben (1919) and applied clinically with favorable results by Haden (1919), Sachs and Belcher (1920) and others, has been complicated by the toxicity (Cushing and Foley, 1920; Foley and Putnam, 1920) and secondary rise in cerebrospinal fluid pressure caused by the substances used for dehydrating the brain. The experiments which will be described in this paper indicate that the latter effect may be avoided by using hypertonic sucrose intravenously.

A secondary rise of intracranial pressure from oral administration of sodium salts (200 cc. of 19 per cent Ringer's solution) was seen by Ebaugh and Stevenson (1920) in a patient whose intracranial pressure changes were followed by placing an inverted tambour over the area of bone defect remaining from a subtemporal decompression. The secondary rise, now commonly known to follow the administration of hypertonic sodium chloride by mouth or by vein, was a constant sequence. With saturated

¹ Medical Fellow of the National Research Council.

sodium sulphate by mouth, the pressure was effectively reduced throughout the period of observation lasting 9 hours. With 200 cc. of 30 per cent glucose injected intravenously, the drop in pressure was small; on the other hand, no secondary rise was seen after $8\frac{3}{4}$ hours. Experimental and clinical evidence has since shown that glucose also produces a definite secondary rise in pressure (Browder, 1930; Milles and Hurwitz, 1932; Jackson, Kutsunai, Leader and Joseph, 1933; Masserman, 1934), an effect which was missed by earlier investigators primarily because their experiments were not continued over a sufficiently long period after the injections were made. This secondary effect indicates that the giving of hypertonic glucose for the reduction of intracranial pressure may be inadvisable, since the treatment at one stage aggravates the condition which it is intended to relieve. The tendency has therefore been to discard intravenous therapy (Dandy, 1933) and to depend upon methods which do not produce a secondary rise in pressure. Fay (1924, 1925) has recommended the use of magnesium sulphate by mouth or by rectum, together with restricted fluid intake. Although this eliminates the secondary rise in pressure, the method leads to vomiting or purging and, as pointed out by Fay himself, the dehydration which it produces may be dangerous when the patient's condition is complicated by hemorrhage or shock.

Hughes and Laplace (1930) reported that the cerebrospinal fluid pressure in dogs could be reduced for 3 to 4 hours by intravenous injection of 25 per cent sodium arabinatate solutions. To our knowledge, sodium arabinatate has never been used clinically, probably because its advantages do not warrant the trouble involved in its preparation.

Of all the hypertonic solutions which have been tested as intravenous agents for reducing the cerebrospinal fluid pressure, concentrated glucose has come to be regarded as the most satisfactory (Howe, 1925), although, as pointed out above, it has more recently been shown to produce a secondary rise in pressure. This drawback can probably be attributed to the comparative freedom with which glucose passes from the blood into the spinal fluid. Since changes in the glucose level in the blood are reflected, after a lag, in the spinal fluid (Fremont-Smith, Dailey, Merritt, Carroll and Thomas, 1931), intravenous injection of hypertonic glucose will raise the concentration of sugar in the spinal fluid (Gregersen and Wright, 1935) and increase the osmotic pressure of the latter. Subsequently, when the sugar level in the blood falls, this increase in osmotic pressure presumably causes the accumulation of fluid and the recurrence of high pressure in the ventriculo-subarachnoid system. The degree to which the glucose diffuses into the brain itself has not been determined. Other conditions remaining unchanged, the abnormal pressure is eventually relieved (Masserman, 1934) by the diffusion of glucose out of the spinal fluid and by the utilization of the sugar by surrounding tissues.

It is clear from the above considerations that, for the reduction of cerebrospinal fluid pressure and intracranial tension, a substance is desired which: 1, is non-toxic when given intravenously in high concentrations; 2, produces rapid reduction in the pressure of the cerebrospinal fluid; 3, maintains the period of low pressure for several hours; and finally, 4, does not cause a secondary rise in pressure. To be effective in rapidly drawing fluid from the tissues as indicated by 2, the substance must increase the osmotic pressure of the blood markedly. Conditions 3 and 4 require that it should not leave the blood, or at least that it should not diffuse to a significant degree into the brain or spinal fluid.

From the work of Keith (1924) and Keith and Whelan (1926), and others, as well as from the experience which one of us (M. I. G.) has had with 50 per cent sucrose solutions given intravenously to produce rapid dehydration in dogs, sucrose appears to satisfy the first requirement. This fact, together with the observations of Keith, Wakefield and Power (1932), that in man sucrose is excreted rapidly and completely, indicated that it does not enter the tissues to any great extent, and suggested that it might satisfy requirements 3 and 4.

METHOD. Except for some preliminary experiments on cats, we used dogs exclusively. All animals were deprived of food and water for 15 hours before the experiment.

Ether, dial (Ciba) and sodium amytal (Lilly) were tried as anesthetics. Ether was unsatisfactory for long experiments. Dial caused a troublesome nasopharyngeal exudate, and a gross increase in the cells of the spinal fluid. For example, a dog was given 0.7 cc. dial per kgm.; $7\frac{1}{2}$ hours later the spinal fluid obtained by cisternal puncture contained 1850 white cells per cu. mm. Sodium amytal was used in the majority of experiments. Fifty to 60 mgm. per kgm., dissolved in distilled water to make a 10 per cent solution, were injected intraperitoneally; when increased respiration or slight movements of the legs occurred, small additional doses (0.1 to 0.25 gm.) were given. This anesthetic did not produce any large increase in the number of cells in the spinal fluid. Cisternal puncture in a dog which had been under sodium amytal for 12 hours disclosed a crystal-clear cerebrospinal fluid at 135 mm. pressure, with 20 white cells and 110 red cells per cu. mm. In all our experiments except one, the repeated doses of amytal given to prevent shivering or bodily movements had no apparent effect upon spinal fluid pressure. In two dogs, however, shivering could not be prevented without greatly depressing the respiration.

Prolonged measurements of the cerebrospinal fluid pressure require strict asepsis in order to avoid the pressure changes attending meningitis (Hughes and Laplace, 1930). The skin over the back of the neck was incised and draped with sterile towels; arterial (femoral), venous (femoral) and tracheal cannulae were introduced under aseptic precautions. (The

tracheal cannula was necessary since the mouth was held closed in such a way that the tongue at times interfered with respiration.) After the sterile operation, the dog's head was fixed firmly in a Brodie head holder.

Sucrose (Merck), 50 per cent solution in distilled water, boiled for 5 minutes in a sterile flask, was injected through the femoral venous cannula from a sterile buret, usually at the rate of 5 cc. per minute. In a few experiments this rate of injection was varied in order to determine its effect upon the spinal fluid pressure. Before being injected the solution was warmed to approximately body temperature.

For measuring spinal fluid pressures, we employed a simple 1 mm. bore U-shaped glass tube filled with Ringer's solution. With this manometer, a 100 mm. pressure change involved the displacement of less than 0.08 cc. of fluid.

An 18-gauge needle was used for cisternal puncture. It was fitted on an ordinary T-shaped, three-way metal stopcock which led below through a short piece of rubber tubing to the short arm of the manometer. The outlet of the stopcock opposite the needle was closed with a brass plug, drilled for a watertight fit with a wire obturator, which was bevelled at the end and of such length that when thrust in as far as possible its tip was flush with the tip of the needle. After the cisterna magna was punctured, the obturator was withdrawn to a point which just permitted the stopcock to be turned, connecting the needle with the manometer. This method prevents the loss of any spinal fluid before the pressure is read. Capillary attraction in the manometer was found to be 2 cm.; the manometer scale was therefore set so that its zero mark was 2 cm. above the needle. A fall of 3 cm. in the manometer fluid was sufficient to fill the needle after withdrawal of the obturator. Allowance was made for this volume of fluid displaced by the obturator by filling the manometer to a point 3 cm. above the estimated cerebrospinal fluid pressure (110 to 120 mm. in most dogs) before the puncture was made. *All parts of the pressure-recording system, as well as the Ringer's solution used in the manometer, were sterilized carefully before each experiment.*

The pressure was read at least every two minutes. The readings were always taken at the height of the expiratory rise in pressure. Free respiratory and pulse variations were accepted as indications of an open connection with the cisterna. The cerebrospinal fluid pressure was observed for an hour or more before sucrose was injected, and for 10 to 13 hours subsequently. At the conclusion of the experiment, spinal fluid was removed and red and white cell counts made.

A catheter was passed into the bladder at the beginning of the experiment, and urine measured every 15 minutes. Rectal temperature, heart rate and respiration were noted every 15 minutes. Body temperature was controlled with a heating pad, or with a fan and wet towels on hot days.

Continuous blood-pressure records were obtained from the femoral artery with the use of a large cannula, 5 per cent sodium citrate solution, a mercury manometer and a slowly moving kymograph. In 3 experiments a Hürthle membrane manometer was tried; and in a few experiments the taking of the blood pressure (see figs. 2, 5 and 6) was omitted altogether in order to be sure that loss of small quantities of blood or injection of small quantities of citrate from the wash-out system were not affecting the results, and also because the manipulation necessary for washing out the arterial cannula sometimes disturbed the animal enough to cause changes in cerebrospinal fluid pressure.

EXPERIMENTS AND RESULTS. Twelve experiments were done on cats and 31 on dogs. In addition, 3 dogs were discarded at the beginning of the experiment because of the appearance of blood in the cerebrospinal fluid.

Control experiments. Four control experiments were carried out on dogs under sodium amytal. These experiments all showed a gradual rise in the cerebrospinal fluid pressure of 30 to 45 mm. Ringer's solution during the 10 to 11 hours of observation. This is in agreement with the experiments of Hughes and Laplace (1930), who found that with sodium amytal anesthesia "when any change in the original pressure occurred, it was in the positive direction." Figure 1 shows the most irregular of these controls and is selected to illustrate some of the causes likely to produce variations in spinal fluid pressure. It may be seen that there are several sharp peaks of short duration associated with the manipulation of the animal (intra-peritoneal injections, washing out arterial cannula, etc.). These variations in pressure were to some extent eliminated in later experiments by more careful handling, particularly when the anesthesia was light and additional amytal was being injected intraperitoneally. We found no indication that the pressure varied with the depth of anesthesia, unless the anesthesia was so light as to allow shivering and bodily movements or so deep as to affect the respiration to a marked degree (see p. 91).

Spinal fluid removed at the beginning of this experiment was clear, but contained 200 red cells per cu. mm. In most of the experiments 200 to 300 red cells per cu. mm. were present in the fluid removed at the time of puncture. These may have entered the needle as it passed through the muscles, or they may have come from the cisterna magna. Whenever a vessel of any size was injured there was an immediate sharp rise in pressure and bloody spinal fluid soon appeared in the rubber tubing and the manometer.

In the experiment illustrated in figure 1, six hours after the cisterna magna was punctured, there were 150 white cells per cu. mm., and no red cells in the spinal fluid; at the end it contained 450 white cells per cu. mm. In contrast to these results, it may be well to mention that in our early cat experiments, where no aseptic precautions were taken, we frequently found

several thousand white cells per cu. mm. in the spinal fluid. An experiment described above (p. 84) indicates that the increase in white cells in the fluid was not due to amytal alone. The slow steady rise of pressure seen in figure 1 may have been associated with irritation of the meninges, due to the presence of the needle (Grant, 1929). We do not believe it can be ascribed to infection, in view of the careful aseptic technique employed. *The gradual rise in pressure observed in control experiments should be kept in mind in evaluating the changes in pressure under the conditions of the following experiments.*

Sucrose experiments. Results obtained from 160 cc. 50 per cent sucrose given intravenously in 30 minutes to a dog weighing 13.3 kgm. (6 gm. sucrose

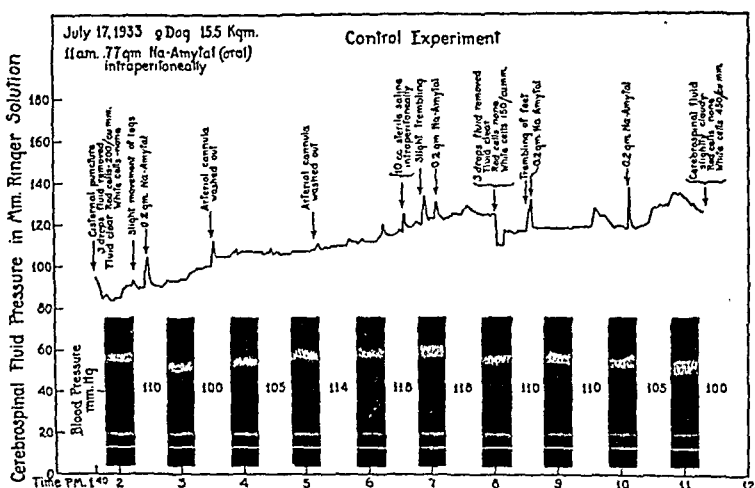


Fig. 1. Showing the gradual rise of cerebrospinal fluid pressure which occurred in all control experiments under sodium amytal anesthesia.

per kgm.) are set forth in figure 2. Control readings obtained for 67 minutes prior to injection showed an average cerebrospinal fluid pressure of 78 mm., with a maximum variation of less than 10 mm. during the whole period. Injection of sucrose was attended by a rise of 15 mm., but 20 minutes after the injection was completed the spinal fluid pressure had fallen to less than 30 mm., i.e., 50 mm. below the original normal level. The pressure remained low for 4 hours and 40 minutes, and although it returned for an hour to the original level, it did not reach the height that a control experiment would probably have displayed by this time. Except for the period of light anesthesia from 9:10 to 9:30 p.m., all pressure readings until the end of the experiment, 12 hours and 15 minutes after the injection was over, were below those observed before sucrose was given. There was no indication whatever of the beginning of a secondary rise in pressure.

Unless the sucrose was administered rapidly a rise in cerebrospinal fluid pressure with injection was the exception rather than the rule. Even so, a rise was not invariably produced by rapid injection. In one experiment 26 cc. in 2 minutes led to a momentary increase of 25 mm.; but in that shown in figure 3, 40 cc. in one minute and a half was followed immediately by a sharp drop of 60 mm. When the rate was adjusted at 5 to 6 cc. per minute from the start of the injection, the pressure customarily remained practically unchanged for 10 to 12 minutes and then began a slow fall which changed to a rapid drop as soon as the injection was stopped. On several occasions it was found that the pressure could be kept constant during the injection by increasing the rate at which sucrose was given, indicating that a balance was being maintained between the effect of

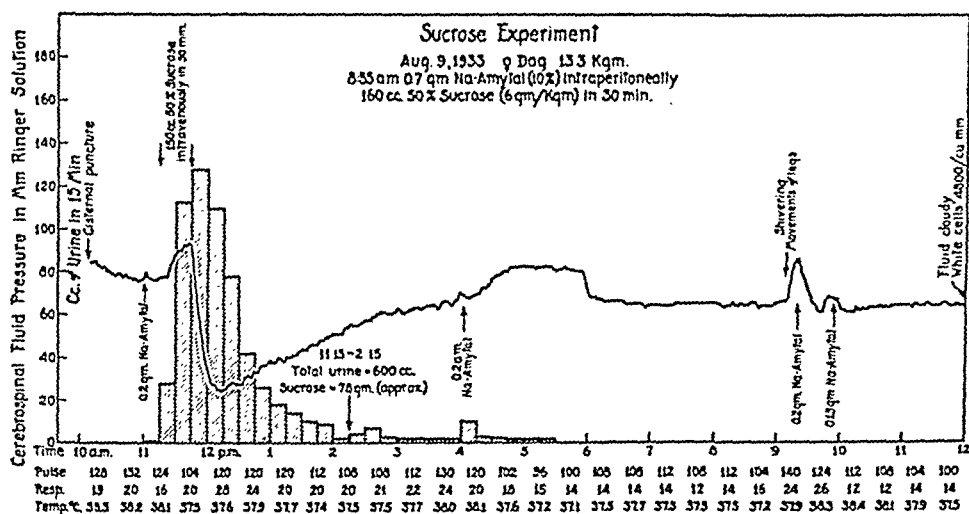


Fig. 2. Reduction of cerebrospinal fluid pressure resulting from the intravenous injection of 6 grams sucrose per kilogram body weight.

increased osmotic pressure of the blood and the effect of increased venous pressure. On no occasion did we observe sudden toxic effects such as those described for sodium chloride by Weed and McKibben (1919) and others. When given slowly sucrose produced no marked change in the heart rate or blood pressure, although there may be a moderate rise in the blood pressure during the period of hydremia which follows the injection (see fig. 3).

From figure 2, it may be seen that 600 cc. of urine were eliminated in 3 hours after the beginning of the sucrose injection, whereas only 160 cc. of fluid were injected. The animal, therefore, lost no less than 440 cc. of body fluid, equivalent to 3.3 per cent of the body weight. Urine passed during active diuresis contained about 78 grams of sucrose, calculated from the difference in reducing material (Benedict's quantitative method) before

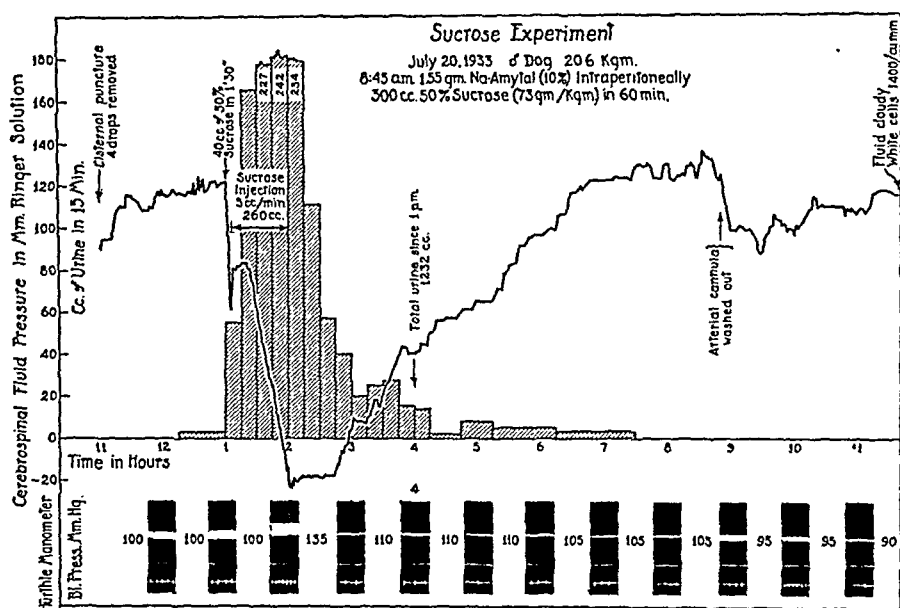


Fig. 3. Injection of 7.3 grams sucrose per kilogram body weight. The spinal fluid pressure dropped to -20.

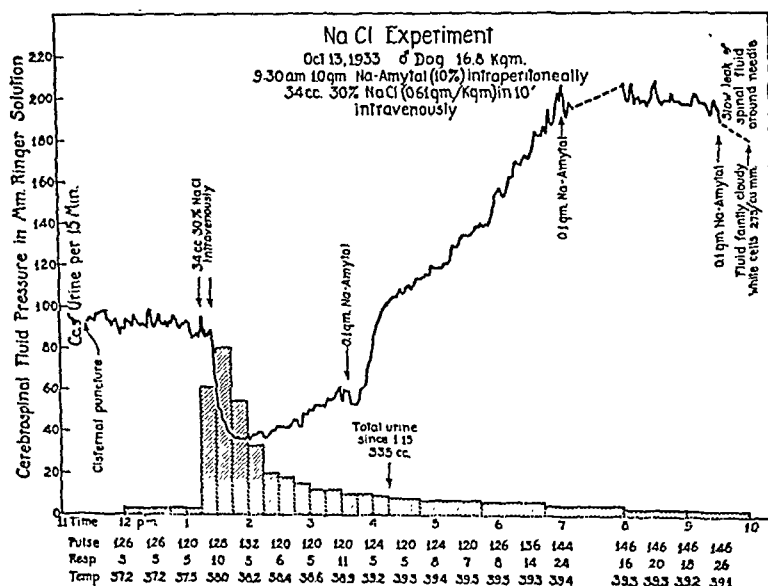


Fig. 4. The temporary fall and subsequent large increase of spinal fluid pressure after the intravenous injection of 0.61 gram sodium chloride per kilogram body weight.

and after hydrolysis. The agreement here between the amount of sucrose injected (80 grams) and the amount excreted is deceiving, for the collections and analyses of urine samples were not accurate enough to give more than

approximate results. Moreover, sterilization of the sucrose solution by boiling may have concentrated it somewhat.² Nevertheless, the experiment serves to show that a large proportion of the sucrose is eliminated by the kidney within 3 hours after injection, taking with it almost 4 times as much fluid as was given in the injection. Under these circumstances, it is not surprising that a secondary rise in cerebrospinal fluid pressure fails to appear, for the agents necessary to produce it, namely, sucrose and water, have both been excreted.

Spinal fluid removed at the end of the experiment contained 4800 white cells per cu. mm. It is interesting that the fluid pressure at the end was lower than the original control pressure, in spite of the evidence of meningeal exudation.

Striking results were also obtained with injection of only 3 grams of sucrose per kilogram of body weight. This is illustrated by the following experiment: the normal pressure for a period of one hour before the injection was about 125 mm. Ringer's solution, with a maximum fluctuation of 11 mm. Twenty-six cubic centimeters of sucrose given in 2 minutes raised the spinal fluid pressure momentarily, but in 6 minutes there was a fall to 90 mm. One hundred cubic centimeters were then given at the rate of 6 cc. per minute, and the pressure continued to fall to 42 mm., i.e., 83 mm. below the level at the start. During 12 hours of observation the pressure never returned to the original control level. Seven hours and 20 minutes after the injection the pressure was still 10 mm. below normal; and 11 hours and 45 minutes after injection it was 20 mm. below the original. Fluid removed at this time contained 150 white cells per cu. mm. Blood-pressure records were taken intermittently. As in the experiments of Weed and Hughson (1921), it was evident that the fall in spinal fluid pressure was not associated with a drop in the blood pressure.

Figure 3 illustrates the results obtained with 7.3 grams of sucrose per kgm. (300 cc. 50 per cent). Four drops of spinal fluid removed at the time of cisternal puncture contained 4 white cells and no red cells per cu. mm. The initial pressure was 90 mm., but it soon rose, and during the last hour of the control period remained at about 120 mm. Forty cubic centimeters of sucrose given in 1½ minutes produced no rise in spinal fluid pressure; on the contrary, there was a sharp drop of 60 mm. in 4 minutes. (The blood pressure fell from an average of 100 mm. Hg to 70, but rose again in 5 minutes. Such a transitory drop in blood pressure was seen in only 2

² The probability is that more than 80 grams of sucrose were actually injected, for Keith, Power and Peterson (1934) have recently shown that in the dog only 70 to 80 per cent of the sucrose given intravenously can be recovered from the urine; this amount appears within 2 to 3 hours. What happens to the rest of the sucrose is not known. In man (Keith, Wakefield and Power, 1932), sucrose is quantitatively excreted.

experiments where the initial injection was made very rapidly.) The remaining 260 cc. of sucrose were given at 5 cc. per minute as indicated on the graph. At the end of the injection, the spinal fluid pressure was -24 mm., a fall of 144 mm. It rose gradually, reaching the original level $5\frac{1}{2}$ hours after the injection was started. For two hours the pressure was normal or slightly above normal, but then maintained a subnormal level for the rest of the experiment, which lasted another 3 hours.

The animal eliminated 1232 cc. of urine in 3 hours after the injection; 932 cc. more fluid were excreted than injected. The dog therefore lost 4.5 per cent of its total body weight in excreting the injected sucrose.

In only 3 other sucrose experiments did the spinal fluid pressure after injection ever exceed the original level. In one of these, autopsy showed that the needle had been thrust into the medulla. In another, a secondary

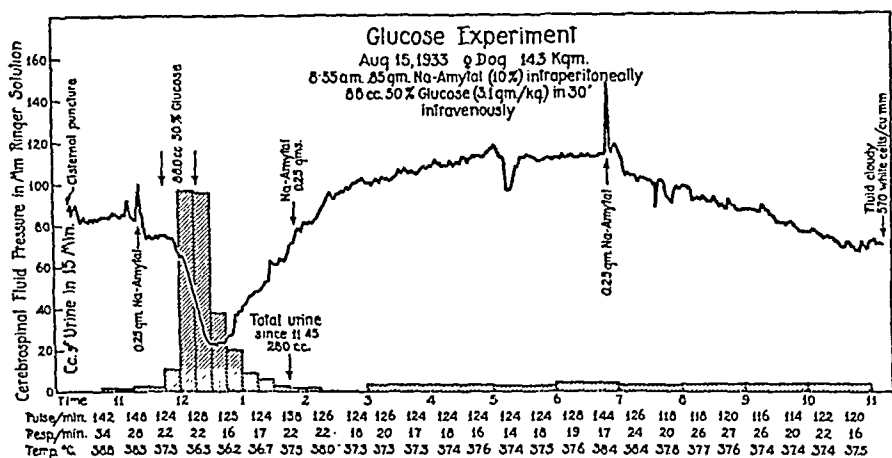


Fig. 5. The fall and secondary rise of cerebrospinal fluid pressure induced by the intravenous injection of 3.1 grams glucose per kilogram body weight.

rise of 40 mm. coincided with deep anesthesia and very slow respiration (2 to 3 per minute); a little artificial ventilation promptly caused the pressure to fall. Anoxemia and not sucrose may be held responsible for this increase in pressure (Dixon and Halliburton, 1914). In the third experiment the rise was temporary and so clearly associated with the giving of amytal that one can hardly attribute it to the sucrose. None of our experiments have, therefore, shown that sucrose leads to a secondary rise of pressure which exceeds the original.

The largest dose of sucrose given in any of our experiments was 11.6 grams per kgm. When that amount was injected, the cerebrospinal fluid pressure dropped from 100 to -60 mm., the lowest pressure we ever obtained with sucrose.

Sodium chloride and glucose experiments. Two experiments were done

with sodium chloride in 30 per cent solution for the purpose of comparing its effect with that obtained from sucrose under identical experimental conditions. In one of these (fig. 4) 0.61 gram sodium chloride per kgm. body weight were given. The cerebrospinal fluid pressure was reduced for only $2\frac{1}{2}$ hours, and within 6 hours after the injection it had risen to 200 mm., i.e., 110 mm. above the pressure observed during the control period. This high pressure was maintained for $3\frac{1}{2}$ hours until fluid began to leak out around the needle in the cisterna magna. In the other experiment, in which 1.64 grams per kgm. were injected, the secondary rise in pressure was equally striking, despite the excretion of 660 cc. of urine in 3 hours. At the conclusion of the experiments, the spinal fluids contained respectively 875 and 500 white cells per cu. mm.

The results obtained on a dog given 3.1 grams of glucose per kgm. in 50 per cent solution are presented in figure 5. After a drop in cerebrospinal fluid pressure lasting 2 hours, there was a rise of 30 to 40 mm. above the control level. Secondary rises were similarly obtained in two other glucose experiments.

DISCUSSION. *Toxicity.* In consideration of this question it must be borne in mind that any hypertonic solution, no matter how inherently non-toxic the substance itself, may have harmful effects if given in sufficiently large quantities or very rapidly. Münzer (1898), for instance, in his study of the toxicity of salts, included for comparison some experiments with dextrose, and demonstrated that this harmless sugar as well as the salts caused death in his rabbits when given in sufficiently large amounts. It is clear, therefore, that although a substance may not have outright toxic effects there is a limit to which the body can tolerate the osmotic disturbances produced by it. The doses of sucrose which have been employed in the above experiments on dogs (3 to 7 gm. per kgm. body weight, except one experiment with 11.6 grams per kgm.), and which effectively reduced the cerebrospinal fluid pressure, were only $\frac{1}{6}$ to $\frac{1}{2}$ as large as the doses which Keith (see p. 84) and one of us (M. I. G., unpublished results) have repeatedly used on dogs and found harmless. As stated above, the solution was usually given at the rate of 5 cc. per minute, although in one experiment (fig. 3) 40 cc. in $1\frac{1}{2}$ minutes had no visible effect except a sudden fall in the spinal fluid pressure. Keith (1933, private communication) has informed us that he has injected 30 per cent sucrose into human subjects in 25 to 30 cases without observing any harmful effects. One of us (L. T. B.) received 3 grams per kgm. body weight (432 cc. 50 per cent solution) intravenously in 2 hours. During the injection this subject became aware of dryness of the mouth and thirst, which could be relieved by sucking a lemon. Later, he noted also a sense of fatigue, malaise and aching of the calf muscles if he exercised; all these symptoms were identical with those experienced by the same subject when dehydrated in other ways (3 days without water,

20 grams of sodium chloride by stomach tube), and disappeared completely upon taking fluids. It seems, therefore, that the only symptoms which were produced by sucrose are directly referable to the withdrawal of water from the body rather than to any specific toxic effect of the sugar. It is not known how rapidly sucrose may safely be given intravenously in man.

Mechanism. ✓ The effect which hypertonic sucrose, glucose or sodium chloride given intravenously and magnesium sulphate given by mouth have upon cerebrospinal fluid pressure is of course only one part of their physiological action. Attention is drawn to certain features of the general disturbance which indicates how the pressure changes are produced.

✓ The immediate result of introducing any hypertonic solution intravenously is an increase in the fluid volume of the blood greater than the volume of the solution injected (von Brasol, 1884; Klikowicz, 1886; Lazarus-Barlow, 1895; Leathes, 1895; Kinsman, Spurling and Jelsma, 1928; Keith, 1924; H. P. Smith, 1925; and others). The hydremia is due to withdrawal of water from the tissues (Tashiro, 1926; Baer, 1926; Skelton, 1927; Ernst, 1930) into the blood, a response to the increased osmotic pressure of the latter. The volume of fluid taken up by the blood is said to be directly proportional to the increase in molar concentration resulting from the injection (Kinsman et al., 1928); but it is apparent that the rate at which the injected substance diffuses into the tissues must be taken into consideration since the movement of fluid into the blood will cease as soon as osmotic equilibrium is established. The osmotic effect of such substances as sodium chloride and glucose, which pass through the capillary wall with relative ease, is greatly curtailed by diffusion.

During the period of hydremia following the administration of sodium chloride, diuresis is facilitated by the high level of salt in the blood and by the dilution of the plasma proteins with water drawn from the tissues (Cushny, 1926; Baird and Haldane, 1922); but diuresis fails to remove more than a fraction (35 to 60 per cent) of the sodium chloride (Münzer, 1898; Padtberg, 1910; Baird and Haldane, 1922), the remainder being stored mainly in the skin (Padtberg, 1910) with less water than is required to dilute it to isotonicity. It is interesting to note that Skelton (1927) found less water in the muscles but more in the skin after hypertonic sodium chloride injections. The storage depots for salt take up and hold water at the expense of other tissues. Moreover, after large quantities of salt have been taken by mouth, ingestion of water produces partly diuresis and partly edema of the tissues in which the excess salt is stored (Baird and Haldane, 1922).

These observations regarding the movement of salt in the body provide an explanation for the temporary fall and subsequent rise in cerebrospinal fluid pressure when hypertonic sodium chloride is injected intravenously

or given by mouth. While the blood is taking up water, it is also losing salt into some of the tissues, which subsequently demand water because of the high osmotic pressure bestowed on them by the salt. That the brain shares in the storage of salt under these circumstances is shown by the observations of Foley and Putnam (1920), that the administration of water produces a much more marked and prolonged rise in cerebrospinal fluid pressure if the animal has been given hypertonic saline several hours previously or the day before. ✓

As in the case of other hypertonic solutions given intravenously, 50 per cent sucrose lowers the spinal fluid pressure because it increases the osmotic pressure of the blood; but a combination of circumstances enables it to have a more prolonged effect than either sodium chloride or glucose. It probably remains in the blood for a longer time after injection since its molecules are larger and less diffusible. It is not broken down to any significant extent or utilized (Folin, Trimble and Newman, 1927) as is glucose, nor is it stored in the tissues as is sodium chloride (see above); sucrose is essentially a non-toxic foreign body once it has entered the blood stream. The reduction of spinal fluid volume produced by its osmotic effect is prolonged by a powerful diuretic action which results in rapid elimination from the body of the sucrose, together with the fluid withdrawn from the tissues (see footnote, p. 90).

Magnesium sulphate when given by mouth or rectum draws water into the gut from the blood, which in turn drains fluid from the tissues, including the brain (Fay, 1924). The fall in cerebrospinal fluid pressure caused by magnesium sulphate therefore actually depends upon realizing a large decrease in the fluid volume of the blood. Whereas this may be of little consequence in the presence of a normal blood volume, it imperils the circulation when the blood volume is already low. Sucrose, on the contrary, produces a temporary state of hydremic plethora during the period when the cerebrospinal fluid pressure is falling. Diuresis removes the excess water from the blood stream in from 2 to 3 hours. The final effect upon the plasma volume must of course be determined by the amount of available body water and by the amount of sucrose which is given. Large doses (15 to 18 gm. per kgm.) produce severe dehydration with a 30 to 40 per cent fall in plasma volume (Keith, 1924); but smaller doses of 3 to 6 grams per kgm. produce either no change in plasma volume of normal dogs or at most a 5 to 10 per cent decrease after 5 to 6 hours (Gregersen).

While 50 per cent sucrose injected intravenously reduces the cerebrospinal fluid pressure markedly in normal anesthetized dogs without producing a secondary rise in pressure, its effectiveness for reducing the cerebrospinal fluid pressure in man is not known. Investigation is under way to determine this point.

SUMMARY

The effects of intravenously injected sucrose, glucose and sodium chloride upon the cerebrospinal fluid pressure have been compared under the same experimental conditions.

Spinal fluid pressures were measured aseptically for 10 to 13 hours on dogs anesthetized with sodium amytal. Control experiments all showed a gradually rising pressure during the period of observation (fig. 1). There was also a moderate increase in the number of white cells in the spinal fluid which was not produced by sodium amytal alone.

Intravenous injection of 50 per cent glucose (3.1 gm. per kgm.) and 30 per cent sodium chloride (0.61 gm. per kgm.) reduced the spinal fluid pressure for only 2 to 3 hours; the pressure then rose above the control level, exceeding it by 40 mm. Ringer's solution (glucose) and 110 mm. (sodium chloride) (figs. 4 and 5).

Three to 8 grams sucrose per kilogram body weight, injected intravenously in 50 per cent solution, reduced the spinal fluid pressure for 5 to 8 hours (figs. 2 and 3). The magnitude of the fall of pressure (50 to 150 mm.) was dependent upon the amount of sucrose given and upon the height of the initial pressure. Greater effects were obtained with higher initial pressures. Although observations were made for 12 hours after injection, there was no indication of the beginning of a secondary rise of pressure exceeding the control level, except in 3 experiments. In these, it could be attributed to definite causes other than the sucrose.

Injection of sucrose induced active diuresis lasting about 3 hours, during which the kidneys eliminated about 4 times as much fluid as was given intravenously with the sugar. With doses of 6 grams per kgm. the body fluid lost by the animal represented 4 to 5 per cent of the total body weight.

The mechanisms by which various hypertonic solutions reduce cerebrospinal fluid pressure are compared and discussed. While temporarily increasing the circulating plasma volume, sucrose reduces the spinal fluid pressure without a secondary rise.

CONCLUSION. Hypertonic (50 per cent) sucrose injected intravenously (3 to 6 gm. per kgm.) is effective in reducing the cerebrospinal fluid pressure in normal anesthetized dogs for 5 to 8 hours without causing a secondary rise exceeding the initial pressure.

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THE EFFECT OF INTRAVENOUS INJECTION OF SUCROSE AND GLUCOSE UPON THE REDUCING POWER OF CEREBRO-SPINAL FLUID, BEFORE AND AFTER HYDROLYSIS

MAGNUS I. GREGERSEN AND LILLIAN WRIGHT

From the Laboratories of Physiology in the Harvard Medical School

Received for publication January 16, 1935

The purpose of the following experiments was to determine whether or not intravenously injected sucrose passes into the cerebrospinal fluid. This problem is pertinent to an understanding of why intravenously injected hypertonic sucrose, like hypertonic glucose or sodium chloride, reduces the cerebrospinal fluid pressure, but unlike these two substances, causes no secondary rise exceeding the initial pressure (Bullock, Gregersen and Kinney, 1935). The commonly accepted explanation of the secondary rise has been that the injected substance which produces it diffuses readily into the cerebrospinal fluid and brain; when the concentration in the blood stream falls, the brain remains hypertonic and becomes edematous (Fay, 1924; Masserman, 1934). If this explanation is correct, it seems justifiable to presume that sucrose does not enter the spinal fluid in appreciable amounts. The same conclusion may be urged from a consideration of the excretion of intravenously injected sucrose as contrasted with that of glucose or sodium chloride. The two latter substances are eliminated only in part (50 per cent or less) (Münzer, 1898; Padtberg, 1910), the remainder being stored, at least temporarily, in tissues which may subsequently swell if water is available (Baird and Haldane, 1922). But sucrose appears not to be stored even temporarily (Keith, Wakefield and Power, 1932); the kidneys eliminate most of it during an active diuresis lasting 2 to 3 hours, and consequently, relatively little can be present in the tissues. Furthermore, since the blood-brain and blood-spinal-fluid barriers are notably less permeable than other blood-tissue barriers, only a small fraction of the remaining sucrose would presumably find its way into the brain and spinal fluid. To test this point, the cisternal fluid from dogs, which had been given intravenous injections of sucrose 1 to 3 hours previously, were analyzed for reducing sugar before and after hydrolysis. As will be shown, no positive evidence of sucrose could be found. But when glucose was injected, a marked hyperglycorachia was observed.

METHODS. Twenty-seven experiments were done on 13 dogs which had been kept in the laboratory for periods of from several months to two

years in connection with other investigations. Eight of the animals had double submaxillary fistulae of long standing (1 to 2 years), three were splenectomized, and two had been used for testing the disappearance rates of vital dyes from the blood stream. All dogs were in excellent health and for purposes of the following experiments were regarded as "normal."

Cerebrospinal fluid was obtained by cisternal puncture (sterile) as quickly as possible (2 to 4 minutes) after giving a small dose (0.3 to 0.4 cc. per kgm.) of nembutal intravenously. In every instance the fluid was crystal clear. All samples were promptly centrifuged for 10 minutes at 3000 r.p.m. in order to make sure that cells were not included in the fluid taken for analysis. The fluids were analyzed (triplicate) as soon as possible after collection. With one exception (August 31), a single puncture was made in each experiment. The results were therefore not affected by the increased rate of formation of cerebrospinal fluid, which occurs when the cisterna magna is tapped (Spurling, 1929; Flexner and Winters, 1932; Flexner, 1933a, b).

Intravenous injections were made into the femoral vein with aseptic precautions. The sucrose and glucose solutions, buffered to pH 7.0, were given in 50 per cent concentration.¹ They were warmed to approximately body temperature before injection and given at 10 to 20 cc. per minute, the rate depending roughly upon the size of the animal. The doses ranged from 6 to 12 cc. or 3 to 6 grams per kilogram body weight (see tables 2 and 3).

CHEMICAL METHODS. *Glucose.* Folin's micro-method (1932) for sugar analysis was adopted because it was found to give almost the same results for glucose and invert sugar. The color comparisons were at first made in a Duboseq colorimeter with a yellow light filter, against a 100 mgm. per cent glucose standard diluted in a Greiner tube (Rothberg and Evans, 1923b) to approximately the color strength of the unknown. When known solutions of 25, 50, 100, 200 and 300 mgm. per cent glucose were compared with a 100 mgm. per cent standard in this manner, the results were too high (40 and 65) for the 25 and 50, and too low (170 and 260) for the 200 and 300 mgm. per cent solutions. Perhaps these results may be explained by the fact that Prussian blue is not the only substance present which lends color and density to the solution. Rothberg and Evans (1923a) stressed the importance of using water-clear reagents in order to obtain satisfactory results with their modification of the Folin-Wu method. In the Folin micro-method this condition is not satisfied since the final solution contains, besides Prussian blue, an excess of the yellow potassium ferricyanide and colloidal iron-gum ghatti. When a series of samples ranging from 25 to 300 mgm. glucose are adjusted by dilution to similar shades of

¹ These solutions were kindly supplied by Eli Lilly and Company.

blue, these reagents are obviously present in decreasing concentration as we pass from the 25 to the 300 mgm. per cent glucose solution. It is clear, therefore, that in order to obtain the correct value for any solution, it is necessary always to use a standard which corresponds closely in concentration to the "unknown."

The inconvenience of preparing a series of standards with every analysis was eventually eliminated by measuring the depth of color in terms of optical density with a spectrophotometer. This instrument has been used by Teorell (1931) in a modification of the Fiske-Subbarow method of phosphorus determination; and Urbach (1931-1934) has employed the simpler Pulfrich photometer (Heilmeyer, 1924) in various quantitative chemical methods which depend upon a color reaction. The spectral absorption curve of the Prussian blue solution produced by the Folin micro-method is highest in the region of 670 to 700 $m\mu$ (fig. 1). This indicates that in carrying out glucose determinations with a simple colorimeter it would be better to use a red filter with maximum transmission in the region from 650 to 700 $m\mu$ than to use the yellow picric acid filters commonly employed (Folin and Malmros, 1929; Folin, 1932).

Absorption measurements were made at 670 $m\mu$ with a König Martens spectrophotometer, using a 1 cm. double quartz cell in which the control cup was filled with water. The samples were all diluted to 50 cc. (within 5 minutes after color development), regardless of color strength, and their optical density determined within 30 minutes. No change in color could be found even one hour after color development.

From the analyses of known glucose solutions ranging in concentration from 25 to 300 mgm. per cent, charts have been constructed (figs. 2 and 3) on which the optical density (at 670 $m\mu$) of the ferri-ferrocyanide solution produced in analysis can be translated into milligrams per cent glucose. The color intensity, measured as optical density, is directly proportional to the concentration of glucose, but the line, instead of passing through the origin, intercepts the ordinate at 0.08. The reagents are responsible for this small absorption in samples containing no reducing sugar.

Sucrose. Inversion of sucrose with hydrochloric acid at 80°C. in a water bath was tried but abandoned. The results were quite inconsistent and always low in comparison with the theoretical values, although the period of inversion was varied from 6 to 24 minutes. There seemed to be destruction of sucrose to non-reducing substances in these dilute solutions.

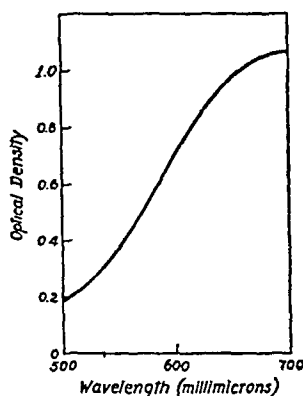


Fig. 1. Spectral absorption curve of the blue-green solution developed by the Folin micro-method for glucose.

The method finally adopted for the determination of sucrose is as follows. Two-tenths of a cubic centimeter of the solution to be analyzed is transferred to 10 cc. of water in a 25 cc. volumetric flask; 1 cc. of concentrated hydrochloric acid (36 to 37 per cent) is added and the mixture allowed to

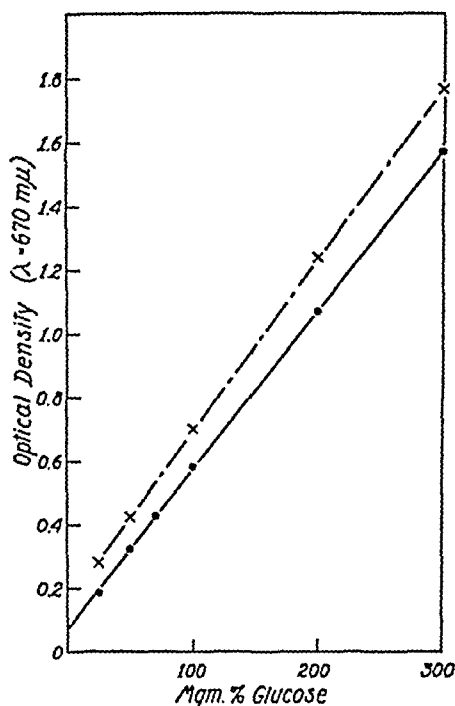


Fig. 2

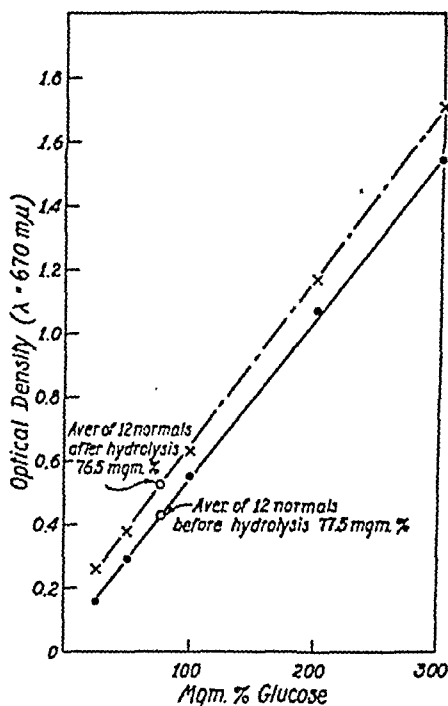


Fig. 3

Fig. 2. Showing the relation between the concentration of glucose and the color developed in analysis by the Folin micro-method. Depth of color expressed as optical density of the solution (1 cm. thick) at wave-length 670 $m\mu$. Plotted from the average results obtained in analyses of 6 series of known solutions (25 to 300 mgm. per cent). •—• = salt-free glucose solutions. X—X = analyses of known glucose solutions containing sodium sulphate or sodium chloride (0.4 normal).

Fig. 3. Showing the effect of the reagents used in hydrolysis upon the reducing power of known glucose solutions (25 to 300 mgm. per cent). •—• = salt-free glucose solutions. X—X = the same solutions analyzed after addition of hydrochloric acid previously neutralized to the end-point of phenolphthalein with sodium hydroxide. Showing also that the increase in reducing power of normal cerebrospinal fluid (dog) caused by hydrolysis does not signify an increase in the reducing sugar.

stand overnight at room temperature (Folin, 1925). The acid is then neutralized to the end-point of phenolphthalein with sodium hydroxide and the flask filled to the 25 cc. mark. Five cubic centimeters of this solution are used in analysis for reducing sugar by Folin's micro-method. After color development all samples are diluted to 50 cc. and as soon as

possible the optical density is determined. The averaged results of the analyses of six series of known solutions of sucrose (25 to 300 mgm. per cent) in water are set forth in figure 4.

To demonstrate the validity of this method for estimating sucrose quantitatively in cerebrospinal fluid, known quantities of sucrose were added to different portions of the same specimen of spinal fluid, and the

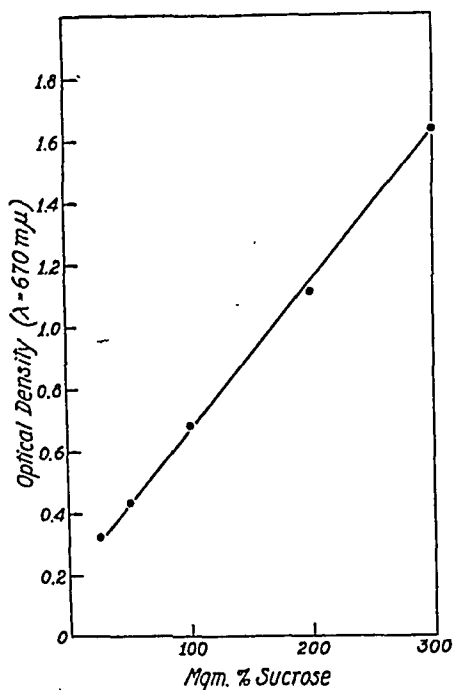


Fig. 4

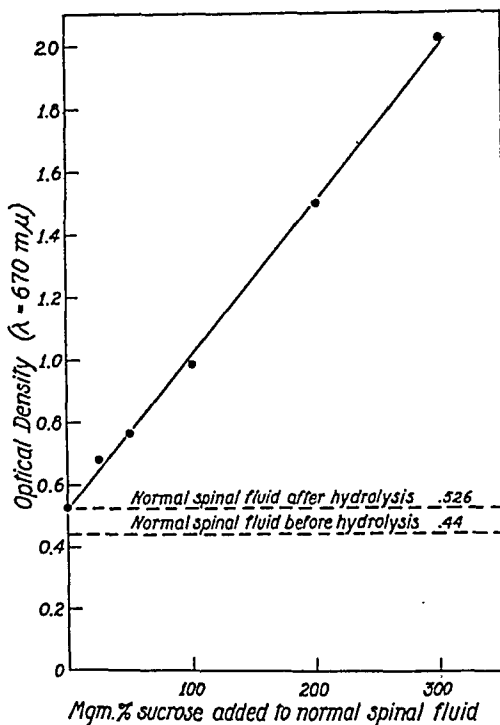


Fig. 5

Fig. 4. Showing the relation between the concentration of sucrose and the depth of color (optical density at 670 mμ) developed in analysis of hydrolyzed sucrose solutions. Average of 6 experiments.

Fig. 5. Determination of sucrose in spinal fluid. Samples of a normal spinal fluid, to which various (known) amounts of sucrose had been added, were subjected to acid hydrolysis at room temperature and analyzed for reducing sugar by the Folin micro-method. The color depth is expressed as optical density at 670 mμ. Average of 6 experiments.

samples analyzed for reducing sugars before and after hydrolysis. The results (fig. 5) show clearly that the reducing power of the spinal fluid is increased by hydrolysis in direct proportion to the amount of sucrose which has been added. A comparison of figures 4 and 5 reveals that the slopes of the lines in these figures are identical.

It was found, however, that even the samples of spinal fluid containing

no sucrose also showed a considerable and consistent increase in the reducing power after hydrolysis, equivalent to about 20 mgm. per cent glucose. From this evidence alone, the hasty and erroneous conclusion might be drawn that normal cerebrospinal fluid contains hydrolyzable reducing material. Further analysis showed that the increase in reducing power is introduced by the reagents used, and that the phenomenon could be duplicated in glucose solutions by merely adding some salt before analysis. If the salt is added after color development has taken place, no change in color intensity occurs. In figure 2 the broken line represents the results of analysis of known glucose solutions to which either sodium chloride or sodium sulphate has been added. In figure 3 the broken line is plotted from results obtained by analysis of three series of known glucose solutions to which the reagents used in hydrolysis (concentrated hydrochloric acid neutralized with sodium hydroxide to the end-point of phenolphthalein) have been added. It is apparent that both procedures lead to a similar increase in the reducing power of the glucose solutions, which again closely approximates the increase obtained by hydrolyzing normal spinal fluid (see below). *The solid line in figure 3, therefore, expresses the relation between the depth of color (optical density at 670 $m\mu$) obtained in analysis and the concentration of reducing sugar (in mgm. per cent glucose), while the somewhat higher broken line gives the same relation after hydrolysis.* The analysis of 12 normal spinal fluids (table 1) before hydrolysis yielded an average optical density of 0.43 and after hydrolysis 0.52. When the latter result is corrected for the effect of reagents, it appears that the glucose value (76.5 mgm. per cent) is nearly the same as that obtained before hydrolysis (77.5 mgm. per cent).

Further support for the opinion that the reagents are responsible for the apparent increase in reducing sugar of normal spinal fluid after hydrolysis was obtained in the following manner: three samples were taken from the same specimen of normal spinal fluid; (a) was analyzed in the usual manner for reducing sugar, (b) was analyzed immediately after the addition of hydrochloric acid previously neutralized with sodium hydroxide to the end-point of phenolphthalein, and (c) was analyzed after treatment overnight with hydrochloric acid. The average results from 6 series of experiments are listed below.

	BEFORE HYDROLYSIS		AFTER HYDROLYSIS
	(a)	(b) neutralized HCl added	(c)
Optical density at 670 $m\mu$	0.433	0.519	0.524

Although neutralized hydrochloric acid is ineffective as a hydrolyzing agent, it evidently produces approximately the same change in color

intensity as the addition of hydrochloric acid under conditions permitting hydrolysis.

RESULTS AND DISCUSSION. The results obtained in 27 experiments are summarized in tables 1, 2 and 3. It appears from table 1 that the glucose concentration in the normal spinal fluid of the dog (fasting) shows considerable variation (65 to 93 mgm. per cent). This is consistent with the findings of others (Grayzel and Orent, 1927; Goodwin and Shelley, 1925; Fremont-Smith, Dailey, Merritt, Carroll and Thomas, 1931; Cohn, Levinson and McCarthy, 1933). In considering significant changes in spinal

TABLE 1
Controls. Normal cerebrospinal fluid (dogs)
Reducing sugar in milligrams per cent glucose*

BEFORE HYDROLYSIS	AFTER HYDROLYSIS	DIFFERENCE
75	79	+4
85	76	-9
90	89	-1
71	69	-2
72	80	+8
70	66	-4
72	63	-9
88	74	-14
93	79	-14
83	81	-2
65	72	+7
69	88	+19
Maximum.....93	89	
Minimum.....65	63	
Average.....77.5	76.5	-1

* The reducing sugar values in tables 1, 2 and 3 have been derived by using figure 3 for converting optical densities obtained in analyses into milligrams per cent glucose.

fluid glucose, this range of normal variations may therefore be a more useful basis for comparison than the actual average figure, 77.5 mgm. per cent.

We have no clear evidence from these experiments of the destruction of reducing substances by hydrolysis such as was observed by Folin and Berglund (1922) in blood, and by Fremont-Smith and Dailey (1925) in spinal fluid. Although the reducing material was less after hydrolysis in more than half the samples (8 out of 12), the average change was only -1 mgm. per cent.

After sucrose had been injected (3 to 6 gm. per kgm.), the reducing sugar in unhydrolyzed spinal fluid was 11 mgm. per cent higher (average than

the normal value taken from table 1. This is probably a real change, since the lowest and highest values obtained, 76 and 104, also showed an increase of the same order. The origin of this rise in spinal fluid glucose

TABLE 2
Sucrose experiments

Fifty per cent sucrose intravenously. Reducing sugar in milligrams per cent glucose.

GRAMS PER KILOGRAM BODY WEIGHT	TIME OF CISTERNAL PUNCTURE AFTER INJECTION		BEFORE HYDROLYSIS	AFTER HYDROLYSIS	DIFFERENCE
	hrs.	min.			
3.0	3	15	80	87	+7
3.0	3	10	85	88	+3
3.0	3		88	90	+2
3.0	2	15	104	110	+6
3.0	2		98	101	+3
3.0	2		83	82	-1
3.6	1		88	91	+3
4.5	1		76	89	+13
6.0	1		93	75	-18
Maximum.....			104	110	
Minimum.....			76	75	
Average.....			88	90	+2

TABLE 3
Glucose experiments

Fifty per cent glucose intravenously. Reducing sugar in milligrams per cent glucose.

GRAMS PER KILOGRAM BODY WEIGHT	TIME OF CISTERNAL PUNCTURE AFTER INJECTION		BEFORE HYDROLYSIS	AFTER HYDROLYSIS	DIFFERENCE
	hrs.	min.			
6.0		15	107		
3.0		50	162		
3.0	1		175	174	-1
3.5	1		187		
	1	30	165	176	+11
3.0	1	20	112		
	1	20	111		
3.0	2		83		
	2		86		

was not investigated. Hydrolysis increased the amount of reducing sugar in 7 out of 9 experiments, but the changes were so small and inconsistent that in our opinion they do not supply positive evidence for the presence of

sucrose. Even if the last experiment were disregarded, the average increase with hydrolysis would be only 4.5 mgm. per cent. From such limited data it is obviously impossible to state definitely whether or not sucrose passes the blood-spinal-fluid barrier.

In the glucose experiments (table 3 and fig. 6) the outcome was in sharp contrast to the results obtained with sucrose. One hour after the injection of 3.5 grams per kilogram (4th expt. in table 3), the spinal fluid glucose was 187 mgm. per cent, i.e., more than twice as great as the extreme upper value found in the normal fluids (table 1). In only one experiment was the concentration of glucose within the normal range. In this case the sample was taken two hours after injection of 3 grams per kilogram. The

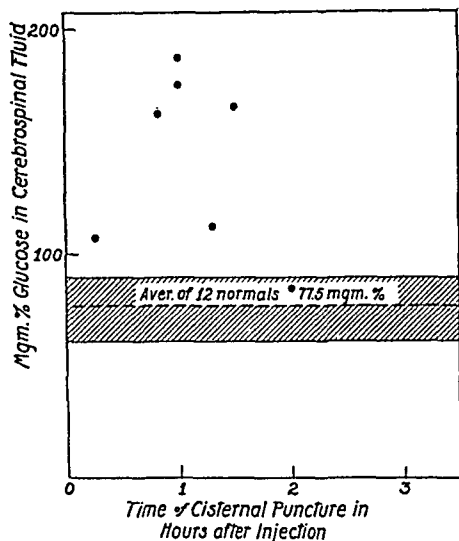


Fig. 6. Solid circles = the sugar concentrations in the cerebrospinal fluid of dogs which have been given hypertonic glucose intravenously. Cross-hatched area = range of glucose concentrations found in 12 normal spinal fluids.

first and last portions were collected separately but the analyses (triplicate) still agreed within 3 mgm. per cent. Specimens from experiments 3 and 4 in table 3 were also tested for reducing sugar after hydrolysis. At the time of the other four experiments the possible interest of the effect of hydrolysis was unfortunately not appreciated. The two results obtained do not indicate any significant change in the amount of hydrolyzable substances present.

It might be suggested that our experiments would be more convincing if control samples of cisternal fluid had been obtained from each animal immediately before the injection of sucrose (or glucose) and compared with samples taken subsequently during the course of the experiments. The objection to such a procedure is that the long period of anesthesia and the

effect of repeated punctures, with removal of fluid, would make the experiments less illustrative of the true changes in spinal fluid occurring in the normal animal. To be sure, the effect of the anesthetic upon the composition of the spinal fluid was not entirely excluded, but it was at least minimized by giving the anesthetic (nembutal) just before (2 to 4 min.) the cisternal punctures were made. Since ventricular pressure influences the rate of cerebrospinal fluid production (Flexner, 1933) and probably also the permeability of the choroid plexus (Spurling, 1929), the results obtained with repeated withdrawal of fluid cannot be regarded as strictly analogous to the effects produced in a closed system which has not been disturbed during the experiment.

From the literature (Flexner, 1934; Cumings and Carmichael, 1934) it is apparent that agreement has not been reached concerning the effect of hyperglycemia upon the concentration of glucose in the cerebrospinal fluid. Munch-Petersen (1930), for instance, claims that hyperglycemia alone does not produce hyperglycorachia. This author found that adrenalin-hyperglycemia, or glucose ingestion combined with adrenalin subcutaneously, was required to raise the spinal fluid glucose 16 to 30 mgm. per cent. On the other hand, a number of investigators, including Goodwin and Shelley (1925), Grayzel and Orent (1927), Fremont-Smith, Dailey, Merritt, Carroll and Thomas (1931), Spurling (1929), and recently Cohn, Levinson and McCarthy (1933), using improved chemical methods, have obtained definite evidence that the spinal fluid glucose concentration rises with hyperglycemia. No doubt some of the conflicting results have been due to differences in chemical methods, the experimental conditions and the animals used. For the purpose of comparing the diffusion of glucose and sucrose into the spinal fluid it is therefore highly significant that our glucose and sucrose experiments were performed under similar conditions and with the same methods throughout.

A question of considerable practical significance is whether or not the blood-spinal-fluid barrier would still display the same degree of impermeability to sucrose after cranial injury (Hoff, 1930). Could hypertonic sucrose be used effectively to break up the vicious circle that is started by such an injury (Cannon, 1901)? In this connection it is important to know whether or not the blood-brain barrier is similar to the blood-spinal-fluid barrier with respect to its impermeability to sucrose.

SUMMARY

In 12 normal spinal fluids obtained from fasting dogs the glucose concentration varied from 65 to 93 mgm. per cent; the average was 77.5. Intravenous injection of 3 to 6 grams of sucrose per kilogram body weight failed to cause a definitely measurable increase in hydrolyzable reducing material in the cerebrospinal fluid (cf. tables 1 and 2). This apparently means that

sucrose does not pass the blood-spinal-fluid barrier. On the other hand, injections of 3 to 3.5 grams of glucose per kilogram produced a marked hyperglycorachia (187 mgm. per cent) within one hour after the injection (table 3 and fig. 6), showing that under similar conditions glucose diffuses readily into the cerebrospinal fluid.

These observations were made with the Folin micro-method for glucose, but spectrophotometric analysis was substituted for colorimetry (Duboseq) in order to avoid the preparation of a series of standards with every determination. The optical density, at 670 $m\mu$, of the blue-green solution produced in the analysis appears to be directly proportional to the sugar content of the sample (figs. 2 and 3).

Evidence obtained indicates that normal cerebrospinal fluid contains no hydrolyzable reducing material. The addition of sodium chloride, sodium sulphate (fig. 1) or neutralized hydrochloric acid (fig. 2) to known glucose solutions increases their reducing power to the same extent as that observed in normal spinal fluid after acid hydrolysis.

CONCLUSION. Sucrose does not appear in definitely measurable amounts in the cerebrospinal fluid of dogs after intravenous injection of 3 to 6 grams per kilogram body weight. Intravenous injection of 3 to 3.5 grams of glucose per kilogram is followed by marked hyperglycorachia.

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THE UPPER LIMIT OF HEARING IN CHIMPANZEE

J. H. ELDER

From the Laboratories of Comparative Psychobiology, Yale University

Received for publication February 19, 1935

Auditory sensitivity, in its broader meaning, is determined principally by the range of audibility (frequency sensitivity) and auditory acuity (intensity sensitivity). An animal with either of these capacities well developed has a definite advantage in responding to his environment. Naturalists and experimentalists are familiar with the difficulty of approaching a group of primates or other animals without being detected, even when visual and olfactory stimuli presumably are absent. If the cues are auditory and yet beyond the human limits of audibility what is the nature of the stimulus? Is it a matter of auditory acuity or of high frequency sensitivity? Certain animals which have been credited with an extremely keen sense of hearing may be sensitive to vibrations of very low intensity or of very high frequency. There is the further possibility that there may be superiority in both respects. We have insufficient experimental data to know to what extent, if at all, the two capacities are functionally related and coexistent. It is clear, then, that a test of auditory acuity alone does not provide a satisfactory description of auditory sensitivity.

The upper limit of hearing in man has been well established, but with exception of the dog and cat it has not been determined for other mammals. Andreyev (1), using the conditioned salivary reflex method, found the limit for the dog to lie between 36,000 and 38,000 c.p.s., or somewhat lower than results of earlier observations (2). Wever (9) found the limit of audibility for the cat to lie in the region of 10,000 to 20,000 c.p.s. Results of Dworkin, Seymour, and Sutherland (3) indicating "that the cat's range extends beyond that of the dog and far beyond that of man" are qualified by the statement of the experimenters that their loud speaker may not have been functioning properly above 20,000 c.p.s. No reliable data have been obtained for the infrahuman primates other than a few observations by Wendt (8) on monkeys. His tests of high frequency sensitivity, given to only one of five subjects, were incidental to a study of auditory acuity. Responses occurred to tones from a Pierce magnetostriction oscillator¹

¹ This was the oscillator used with chimpanzees in the present study. Experimental conditions for the monkeys and chimpanzees differed in a few details.

at frequencies from 16,500 ~ to 33,600 ~. Higher frequencies were not available at the time and the upper limit was not determined.

The aim of the present study was to determine the upper limit of hearing in chimpanzees. Three young chimpanzees and three children were used in the same situation.

SUBJECTS. Two of the chimpanzees used in this experiment, Moos and Bimba, no. 11 and no. 26 respectively in the laboratory series, had been used extensively in previous work on absolute intensity thresholds. The third, Alpha (no. 28), had been used very little experimentally and was without experience in auditory work. Her early history has been described by Jacobsen, Jacobsen and Yoshioka in their study of chimpanzee behavior during the first year (5). Information concerning the characteristics and life histories of Moos and Bimba has been presented in a previous report (4). At the beginning of the investigation the ages of these subjects were: Moos, seven years (hypothetical), Bimba, five years (hypothetical), Alpha, three years and five months.

The three children had no known physical defects and had normal auditory acuity for their ages. Subjects L. W. and H. G. were twelve years old, B. E. was five and one-half years. Audiograms for the children and for two of the chimpanzees are presented in figure 1. Those for the children represent results of the usual clinical tests with the 2-A Western Electric audiometer. Examination of the chimpanzees was made in a similar manner except that a food reward was given for correct responses and steps of one decibel attenuation instead of five were used. The latter difference coupled with the fact that both were highly trained probably accounts for the more uniform appearance of the chimpanzee curves.

APPARATUS AND PROCEDURE. In the preliminary training period of the investigation the 2-A audiometer was used as the source of stimuli. High frequency tones were obtained from a Pierce magnetostriction oscillator (6) (7). In this instrument tones result from the longitudinal vibration of nichrome rods, the frequency of vibration being determined by the length of the rod. An outstanding advantage of this apparatus is the constancy of frequency under uniform electrical and temperature conditions. The rods used were cut to approximate a given frequency and then accurately measured in order to determine the actual frequency. Unfortunately, data concerning intensity of the tones are not available. According to Mr. H. W. Lamson, Engineer of the General Radio Company, the intensity level is well above that of the average minimum intensity for human audibility. Frequencies of 14,400 ~ and 16,500 ~ were of sufficient intensity to be disagreeable for most subjects and could be heard without difficulty several hundred feet from the instrument. The following frequencies, expressed in the nearest 100 cycles, were used as stimuli: 14,400, 16,500, 19,000, 20,700, 22,900, 25,000, 30,500, 33,600, and 37,500

cycles per second. The method employed in the present investigation is essentially the same as that used in my previous work on auditory acuity (4). For convenience of the reader the essential features are repeated here.

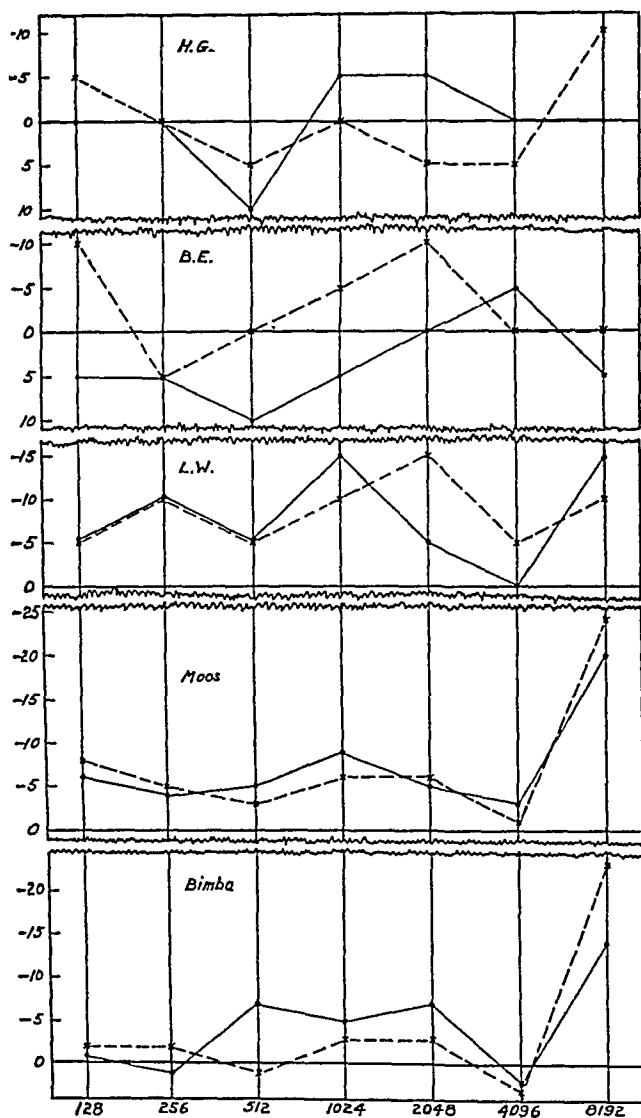


Fig. 1. Audiograms for three human subjects and two chimpanzees. Thresholds for the right ear are indicated by the dots, those for the left by the crosses. The heavy line at 0 represents the average human threshold. Ordinate values are units of intensity above and below the average human threshold, negative values indicating "better" thresholds. Frequencies of complete vibrations are shown along the base line.

Observations were made in a semi-soundproof room with subject and experimenter separated by a one-way vision screen. The subject was

seated at a table facing the screen and directly in front of a food delivery chute and a telegraph key, covered with a hood and sliding panel. The stimulus came from a partially enclosed box 15 to 18 inches from the subject at a point forty-five degrees from the mid-plane in the upper left quadrant. Since it was impossible to keep the subject's head in a fixed position this relationship was not constant although there was little change in distance.

As a preliminary to determination of upper pitch limens each subject was trained to respond to a tone by pressing the key and to refrain from pressing in the absence of a tone. Most of this training was done at a frequency of 8192 c.p.s. with the 2-A audiometer as stimulus source. With Moos and Bimba, because of previous mastery of the procedure, this training was not necessary but as a matter of routine they were required to work through three training periods of twenty trials each with 8192 c.p.s. at a barely audible intensity before passing to the higher frequency tests.

The method of presenting the stimuli after preliminary training was as follows. With the subject seated before the table the experimenter raised the sliding panel, exposing the key, and after three seconds, either presented or did not present a tone. An experimental period consisted of twenty trials, ten of which were without stimuli.

For each correct response the chimpanzees were rewarded with a piece of fruit which dropped into the cup; a false response (response to absence of tone) was unrewarded. For each correct response the human subjects were rewarded with a penny.

RESULTS. None of the subjects used failed to respond to a frequency of 16,500 \sim or less. The frequency at which the formal tests were begun was determined for each subject on the basis of response to several preliminary presentations of successively increasing frequencies. The highest frequency to which a subject uniformly responded was taken as the starting point of the daily test series.

Table 1 shows the total number of trials given to each chimpanzee at various frequencies, the percentage of correct responses, and the number of false responses. Table 2 gives corresponding data for the children. It should be noted that the actual number of stimulus presentations is one-half of the total number of trials and that the percentage of correct responses is based upon stimulus trials only. The non-stimulus trials served as a part of the training against guessing. They are important chiefly as an indication of reliability of the subjects' positive responses. In table 1, for example, Moos was given ten and twenty tone presentations at 25,000 \sim and 27,400 \sim , respectively, and an equal number of trials in which the stimulus was absent. He responded to all of the stimuli and made no responses in the non-stimulus trials. At 30,500 \sim he responded in forty-four of the sixty stimulus trials and also in three of the sixty non-stimulus trials. Ordinarily the total number of trials is a multiple of twenty, the

regular experimental series. The three exceptions, which appear in the records of Moos and Alpha, indicate that an experimental period was interrupted by emotional disturbance (temper tantrum) or complete failure and refusal to work at a particular frequency. Alpha, for instance, after almost complete failure at 27,400 ~ refused to work, not only at this frequency but at all others.

TABLE 1

Responses of chimpanzee subjects to frequencies of 22,900~ to 37,500~

FRE- QUENCY	MOOS			BIMBA			ALPHA		
	Number of trials	Per cent correct	Number of false responses	Number of trials	Per cent correct	Number of false responses	Number of trials	Per cent correct	Number of false responses
22,900				20	100	0			
25,000	20	100	0	20	90	0	20	100	0
27,400	40	100	0	80	70	0	26	23	4
30,500	120	73.3	3	40	30	0			
33,600	111	47.3	2						
37,500	8	0	0						

Percentages of correct responses are based upon the number of stimulus trials only (one-half of the total trials); false responses are responses occurring in non stimulus trials.

TABLE 2

Responses of human subjects to frequencies of 16,500~ to 25,000~

FRE- QUENCY	L. W.			B. E.			H. G.		
	Number of trials	Per cent correct	Number of false responses	Number of trials	Per cent correct	Number of false responses	Number of trials	Per cent correct	Number of false responses
16,500	20	100	0						
19,000	20	90	0				20	100	1
20,700	60	50	5	20	100	0	20	70	0
22,900	20	10	2	40	85	0	60	53	2
25,000				20	0	0	20	0	2

Percentages of correct responses are based upon the number of stimulus trials only (one-half of the total trials); false responses are responses occurring in non-stimulus trials.

Figure 2 is a graphic representation of the performance of all subjects, in which are shown percentages of correct responses at different frequencies and the fifty per cent threshold for each individual. All frequencies lower than those represented on the curves were regularly responded to; there were no indications of the presence of tonal islands or other unusual conditions.

DISCUSSION OF RESULTS. Immediately apparent from inspection of

figure 2 is the fact that the curves for chimpanzees and children form two distinct groups. Two of the animals have records of perfect response at 25,000 ~, a frequency to which none of the children responded. Several human adults, examined briefly in the same situation, never responded to this frequency. In fact, only one of them was able to hear the 22,900 ~ tone; most of them could hear 19,000 ~; while a few of the older subjects failed to hear 14,400 ~. All of the experimental data, as well as the results of these informal tests agree with previous determinations that the upper limit of hearing in man is approximately 20,000 cycles per second. The upper limit for chimpanzees under eight years old, as indicated by tests with three subjects, is near 30,000 cycles per second.

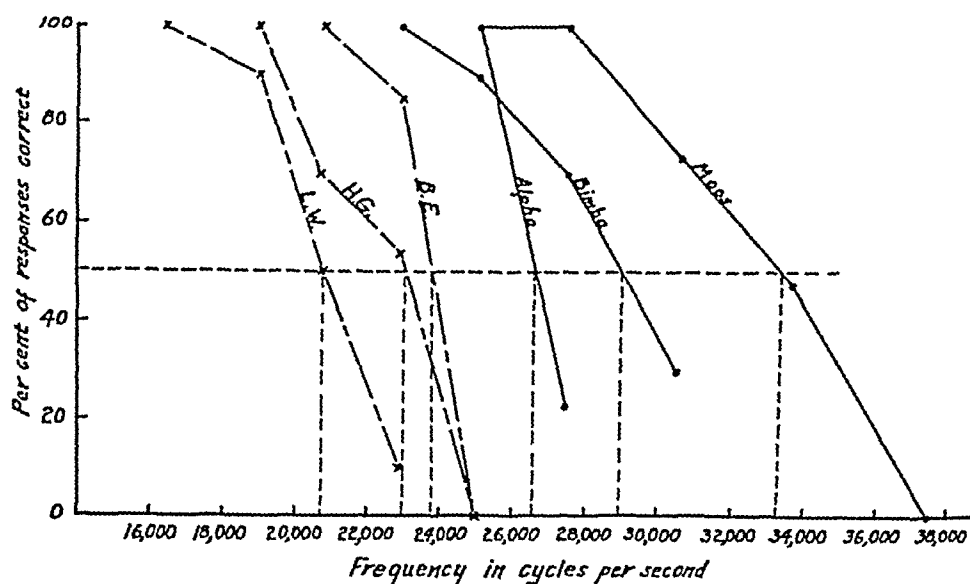


Fig. 2. Upper frequency limens for three human subjects and three chimpanzees. Each limen is indicated by the dotted perpendicular dropped from the intersection of individual curves and the horizontal 50 per cent line..

The factor of practice should be considered in evaluation of the foregoing results. It was stated above that Moos and Bimba were used extensively in auditory acuity experiments while the third subject, Alpha, did not have the advantage of this experience. It is possible that this difference is reflected in the results, probably as the effect of a more serviceable general adaptation as a result of long practice rather than a real change in sensory capacity (no subject had more practice than another with tones above 8,192 ~). Effects of practice, within the present experiment, were negligible in all cases; and the fluctuations from day to day, frequently observed when working with minimum intensities were likewise small.

A comparison of the results presented here with those obtained by Wever for cats reveals some interesting differences. Just how much may be

ascribed to differences in subjects observed and how much to methods employed it is impossible to say. Aside from the fact that the limens for Wever's cats are all lower than those of the chimpanzees, there are large differences in the frequency range over which responses were obtained. The general form of the curves is also different. Whereas the responses of chimpanzees as well as children fell off rather sharply with relatively small increases in frequency, the cats continued to make the "flutter response" at a fairly low level of efficiency over a much broader range.

It is commonly supposed that monkeys, and also chimpanzees, have a keener sense of hearing than man. It is pertinent, therefore, to suggest that this superiority may be determined entirely by sensory capacity for high frequency vibrations. The conclusion needs further confirmation, but we already have the evidence of Wendt (8) that the auditory acuity of monkeys for tones below 8,192 \sim is of the same order as that of man, whereas for tones above 8,192 \sim the monkey excels. The writer (4) has shown that essentially the same relationship holds for chimpanzee and man.

It is believed that valuable results will be obtained from further studies of high frequency sensitivity in primates, especially if such studies can be correlated with morphological and physiological observations.

SUMMARY

Three chimpanzees and three children, trained to respond to a tone by pressing a key and to refrain from response when tone was absent, were tested with nine different tones ranging from 14,400 to 37,500 cycles per second.

The upper limit of hearing (defined as that value of the stimulus to which responses are made in fifty per cent of the presentations) for the chimpanzees ranged from 26,000 \sim to 33,300 \sim , and for the children, from 22,600 \sim to 23,700 \sim .

The conclusion is offered that difference in auditory sensitivity between man and other primates probably is a result of the latter's relative superiority in reception of high frequency sounds.

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THE RESYNTHESIS OF PHOSPHOCREATINE AFTER MUSCULAR CONTRACTION

JACOB SACKS AND WILMA C. SACKS

From the Laboratory of Pharmacology, University of Michigan Medical School, Ann Arbor, Michigan

Received for publication February 15, 1935

Evidence has been presented previously (15b) in support of Fiske's (4) theory that the function of phosphocreatine hydrolysis in muscle is that of buffering the lactic acid formed during contraction under anaerobic conditions. It was shown that in the muscles of rabbits which had been on a diet deficient in base, the amount of phosphocreatine hydrolyzed during a tetanic contraction was such that the pH of the muscle fiber would remain constant. On this basis it might be expected that a similar relationship would be found during the recovery process; i.e., the amount of phosphocreatine resynthesized from its hydrolysis products in a given time should be equivalent, in terms of base removed, to the amount of lactic acid concurrently removed. This relation should hold without regard to whether the lactic acid is removed by oxidation, conversion to glycogen, or diffusion into the blood stream.

The technique used previously readily permits the study of the changes taking place in the muscle itself during the recovery process. Experiments were performed on both cats and rabbits. Cats were used because Imrie and Jenkinson (5) have studied the resynthesis of phosphocreatine in this species. The cats were anesthetized with sodium pentobarbital, the rabbits, with sodium pentobarbital or sodium amytal, and ether. The gastrocnemius muscles were dissected free except for their origins, taking care to leave the nervous and vascular connections intact. The peroneal and tibial nerves were cut just distal to their points of emergence beneath the gastrocnemius. It was thus possible to stimulate the gastrocnemius through the nerve without producing contractions of the other muscles of the leg. The portion of the os calcis into which the tendon of the gastrocnemius is inserted was separated from the rest of the bone. By means of a fairly heavy copper wire wrapped tightly around the tendon just at the enlargement made by its insertion into the bone, the muscle was attached to an isometric lever patterned after that described by Martin, Field and Hall (8). The origin of the muscle was held fixed by a steel drill passing through the condyles of the femur. The drill was mounted in a heavy brass base which was clamped to the table top.

In one set of experiments one gastrocnemius was attached to the isometric lever, put under a few hundred grams tension, and tetanized for 30 seconds. Immediately after the stimulation was ended the muscle was frozen. Less than three seconds elapsed between the end of the stimulation and the completion of the freezing. The resting muscle was frozen as soon as possible after the stimulated one, generally within two minutes. In the

TABLE 1

Resynthesis of phosphocreatine and removal of lactic acid from muscles of cats after contraction

Values expressed as milligrams per cent of P and of lactic acid

RESTING MUSCLE			STIMULATED 30 SECONDS NO RECOVERY			DIFFERENCE			TENSION-TIME IN KGM.-SEC. PER GRAM OF MUSCLE
Inorganic P	Inorganic P plus phospho- creatine P	Lactic acid	Inorganic P	Inorganic plus phospho- creatine P	Lactic acid	Phospho- creatine hydrolysis	Hexose- phosphate formation	Lactic acid formation	
21	87	16	37	59	181	16	28	165	12.7
17	94	15	38	63	206	21	31	191	6.7
23	88	11	51	63	180	28	25	169	14.6
22	87	10	38	63	172	16	24	161	18.3
22	90	10	42	63	204	20	27	194	13.1
23	89	13	50	67	194	27	22	181	10.1
Average						21.3	26.2	176.8	12.6
			STIMULATED 30 SECONDS RECOVERY 60 SECONDS						
21	93	10	32	68	165	11	25	155	9.1
23	97	11	30	70	178	7	27	167	12.3
22	99	8	32	74	136	10	25	128	14.9
21	91	13	21	63	133	0	28	120	11.6
22	87	10	20	57	155	-2	30	145	10.7
25	95	10	30	69	152	5	26	142	17.8
Average						5.2	26.8	142.8	12.7

Lactic acid removed..... 34.0 mgm. per cent

Phosphocreatine-P resynthesized..... 16.1 mgm. per cent

Ratio 2.11

other set of experiments the tendon was cut immediately after the end of a similar tetanus of 30 seconds' duration, and the muscle allowed to recover, in the relaxed state, for 60 or 120 seconds before it was frozen. Here also the resting muscle was frozen immediately after the stimulated one. Determinations of phosphocreatine hydrolysis, hexosephosphate formation, and lactic acid formation were made by the methods previously described (15a). The differences in inorganic P and lactic acid content, above the

resting level, measure the amounts of phosphocreatine resynthesized and lactic acid removed.

The data, given in tabular form, show that there is practically no change in the hexosephosphate content during the first two minutes of recovery. However, the removal of lactic acid and the resynthesis of phosphocreatine from its hydrolysis products do take place in large amount. It is also seen that there is a very close correspondence in the rate at which these two processes are taking place. The ratio of lactic acid removed to phosphocreatine-P resynthesized is close to 2.5 to 1.

At pH 5.6, which Rous (14) has found within the mammalian muscle fiber, the formation of one milligram of phosphocreatine-P from inorganic phosphate and creatine removes base equivalent to 2.5 mgm. of lactic acid. Hence if phosphocreatine resynthesis and lactic acid removal are taking place simultaneously in this proportion, the pH of the system would remain constant. Examination of the data readily permits such an interpretation. The quantities of acid and base removed are equivalent; no matter what absolute amount of lactic acid is removed per minute, phosphocreatine is synthesized from its hydrolysis products in equivalent amount.

Imrie and Jenkinson found that phosphocreatine was resynthesized in cat muscle at the rate of about one-half of one milligram per cent per minute, in contrast to the average value of 16 mgm. per cent found here. They cut the nerves supplying the muscles and allowed an hour or more to elapse between the nerve section and the stimulation. It appears, then, that denervation deranges the mechanism for phosphocreatine synthesis in mammalian muscle.

It would be expected, from the work of Eggleton and Evans (3), that the greater part of the lactic acid which disappears from the muscles in the early part of the recovery period diffuses into the blood stream. In this connection it is necessary to compare the resynthesis of phosphocreatine in mammalian muscle with intact circulation, with the anaerobic resynthesis found by Nachmansohn (13) in isolated frog muscle, under conditions in which the muscle cannot lose lactic acid by diffusion. The fibers, however, can, and in all probability do, lose lactic acid by diffusion into the interspaces. Phosphate ion, on the other hand, apparently does not diffuse from the fiber into the interspaces. This is the interpretation that Eggleton (2) puts on the finding that only 20 to 30 per cent of the water of the muscle is available for free diffusion of phosphate. If some 20 to 30 per cent of the muscle substance is composed of interspaces, then this portion of the lactic acid formed during a tetanus could be expected to diffuse out of the fibers afterward. Then this percentage of the phosphocreatine hydrolyzed during the tetanus must be resynthesized afterward in order to maintain the pH constant within the fiber. Nachmansohn (13) and Lunds-gaard (6c) both find that about 30 per cent of the phosphocreatine hydro-

TABLE 2

Resynthesis of phosphocreatine and removal of lactic acid from muscles of rabbits after contraction

Values expressed as milligrams per cent of P and of lactic acid

RESTING MUSCLE			STIMULATED 30 SECONDS NO RECOVERY			DIFFERENCE		
Inorganic P	Inorganic plus Phospho- creatine P	Lactic acid	Inorganic P	Inorganic plus Phospho- creatine P	Lactic acid	Phospho- creatine hydrolysis	Hexose- phosphate formation	Lactic acid formation
14	96	30	33	60	197	19	26	167
18	95	20	28	68	180	10	27	160
19	102	20	39	75	192	20	27	172
25	101	22	44	80	168	19	21	146
21	90	17	34	60	150	13	30	133
17	85	20	35	56	179	18	29	159
11	99	26	33	61	189	22	28	163
19	90	28	40	69	153	21	21	125
Average						17.7	26.1	153.1
			STIMULATED 30 SECONDS RECOVERY 60 SECONDS					
20	106	23	26	76	158	6	30	135
26	94	33	29	73	167	3	21	134
21	99	16	27	80	117	6	19	101
18	98	20	24	77	121	6	21	101
21	100	21	30	79	156	9	21	135
15	101	27	27	75	160	12	26	133
18	110	21	28	75	161	10	35	140
Average						7.4	24.7	125.6

Lactic acid removed..... 27.5 mgm. per cent

Phosphocreatine-P resynthesized..... 10.3 mgm. per cent

Ratio 2.67

			STIMULATED 30 SECONDS RECOVERY 120 SECONDS					
16	87	20	19	54	143	3	33	123
17	92	12	16	63	108	-1	29	96
18	100	15	21	74	117	3	26	102
19	95	21	19	68	138	0	27	117
15	93	22	19	66	139	4	27	117
18	95	22	18	66	134	0	29	112
Average						1.5	28.5	111.2

Lactic acid removed.....14.4 mgm. per cent

Phosphocreatine-P resynthesized..... 5.9 mgm. per cent

Ratio 2.44

lyzed during a single tetanus is resynthesized anaerobically afterward, and that this resynthesis takes place only during the first 20 or 30 seconds after the end of the tetanus.

In a previous paper (15b) it was stated that this anaerobic resynthesis, which Lundsgaard considers to be the immediate reaction in the recovery process, could be regarded rather as the restoration of pH equilibrium within the fiber, on the assumption that the pH of the frog muscle fiber is the same as that of the mammalian muscle fiber. Without abandoning that assumption it is evident that the explanation given above is independent of the exact pH of the fiber; it requires only that the pH be within the rather wide range over which the hydrolysis of phosphocreatine liberates base.

Since only 30 per cent of the hydrolyzed phosphocreatine can be resynthesized anaerobically, Lundsgaard considers that the remaining, larger, part must wait for oxidative reactions to furnish energy for its resynthesis. The finding that lactic acid removal is the factor controlling phosphocreatine resynthesis accounts for this limitation on the anaerobic process. This also makes it advisable to re-examine the experimental basis for Lundsgaard's hypothesis. If the immediate source of the energy for contraction is the hydrolysis of phosphocreatine, and if recovery consists of the resynthesis thereof, then the muscle must be able to perform this resynthesis at a sufficiently rapid rate to meet the demands of the contraction process. It was found previously (15b) that rabbit muscles produced about 60 mgm. per cent of lactic acid in a 5 second tetanus. From the value of approximately 180 cal. per gram for the heat of formation of lactic acid from glycogen, and approximately 400 cal. per gram of P for the heat of hydrolysis of phosphocreatine, determined in Meyerhof's laboratory (10), the formation of this 60 mgm. per cent of lactic acid should yield sufficient energy for the resynthesis of 27 mgm. per cent of phosphocreatine-P, or at the rate of about 325 mgm. per cent per minute. This is more than 30 times the rate actually found during the first minute of recovery, and more than 50 times the rate found in the second minute. In other words, the Lundsgaard hypothesis requires that the "recovery" process take place with enormously greater velocity *during contraction* than that at which it actually takes place *during recovery*. The data showing the anaerobic resynthesis of phosphocreatine in isolated frog muscles lead to the same conclusion. If 30 per cent of the "recovery" process after a 5-second tetanus requires 20 seconds (Nachmansohn) or 30 seconds (Lundsgaard) then the muscle must recover 13 or 20 times as rapidly during contraction as it is found to do afterward. As for oxidative resynthesis of phosphocreatine, Meyerhof and Nachmansohn (12) found that this is much slower in frog muscle than is the anaerobic resynthesis.

Lundsgaard (6b) found that iodoacetate muscles were capable of per-

forming much more work in oxygen than in nitrogen, at the expense of less *net* phosphocreatine breakdown. Mawson (9) has extended this work and has shown that with added lactate and oxygen very much more work was done than without lactate, and that lactic acid disappeared, probably by oxidation, under these conditions. It is *assumed* that phosphocreatine is hydrolyzed, then resynthesized by the oxidation of some lactic acid, for Lundsgaard has shown (6c) that there is no anaerobic resynthesis of phosphocreatine in iodoacetate muscles. *However, neither investigator has demonstrated that phosphocreatine actually is hydrolyzed during the contraction in oxygen.* The experimental procedure used by Lundsgaard was to stimulate companion iodoacetate muscles, one in oxygen, one in nitrogen, with single twitches at such a rate that the oxygen muscle was able to recover its oxygen by inward diffusion between two successive twitches. Then, when the nitrogen muscle had become non-irritable they were both analyzed for phosphocreatine content. Mawson's finding that lactate and oxygen are unable to prevent the rigor caused by increased carbon dioxide concentration makes it highly improbable that the iodoacetate muscle is capable of even oxidative resynthesis of phosphocreatine.

When it is considered that in iodoacetate muscles phosphocreatine is converted directly to hexosephosphates, the possibility that the hydrolysis of this substance can be the normal source of energy for contraction becomes even more remote. The present data show that the reconversion of hexosephosphate-P to phosphocreatine-P is very slow, relative to the resynthesis of that which has merely been hydrolyzed.

The theory of the sources of energy for muscular contraction presented in the first paper of this series makes it unnecessary to ascribe to phosphocreatine any functions incompatible with these data. Under anaerobic conditions, the normal muscle has two sources of energy available: the formation of lactic acid from glycogen, and the formation of hexosephosphate from glycogen and phosphocreatine. Hydrolysis of phosphocreatine occurs only in such measure as will prevent any change of pH within the muscle fiber. In the presence of adequate oxygen supply, lactic acid is oxidized. In iodoacetate muscles contracting anaerobically, the formation of lactic acid is inhibited, and the formation of hexosephosphate becomes the only source of energy. On this basis the correlation between heat production and phosphocreatine disappearance found in Meyerhof's laboratory (11) is easily understandable.

The work of Clark and his collaborators (1) on the frog heart has led to similar conclusions. They find that under anaerobic conditions lactic acid is formed; the phosphocreatine content, which is low to begin with, falls off very rapidly, and the lactic acid first formed passes into the surrounding Ringer's solution in neutral form. Activity continues until, in the presence of alkaline buffers, lactic acid precursors are exhausted; or, in

the absence of buffers, until the acidity reaches the point at which lactic acid formation is inhibited. Iodoacetic acid, they find, "is exerting a highly specific effect on some process or structure which is required only during anaerobic activity," presumably lactic acid formation.

Margaria, Edwards and Dill (7) also arrive at the same conclusions concerning the rôles of lactic acid and oxygen in muscular exercise in man. However, the present data furnish only partial confirmation of their interpretation of the "alactacid" oxygen debt. They find that the portion of the total oxygen debt which is rapidly paid is not due to lactic acid, and may be due to phosphocreatine hydrolysis. Such an interpretation is easily possible from the present data. However, that portion due to hexosephosphate formation is evidently paid very slowly, at least in the early part of the recovery period during which hydrolyzed phosphocreatine is being restored. Nothing in their data can be found to represent this portion of the "alactacid" oxygen debt, although it may be as important quantitatively as the part due simply to hydrolysis.

SUMMARY AND CONCLUSIONS

1. The early stages in the recovery process after muscular contraction have been studied in the muscles of cats and rabbits.

2. Lactic acid removal and phosphocreatine resynthesis are found to proceed *pari passu* in the early stages of recovery.

3. The amount of phosphocreatine resynthesized in a given time is found to be equivalent, in terms of base removed, to the amount of lactic acid concurrently removed.

4. The resynthesis of phosphocreatine from its hydrolysis products after contraction is found to proceed at a rate only a small fraction of that required by the Lundsgaard hypothesis.

5. The reconversion of hexosephosphate-P to phosphocreatine-P is found to be quite slow during the early part of the recovery period.

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CALORIGENIC ACTION OF FAT AND CARBOHYDRATE IN PANCREATIC DIABETES

G. C. RING

From the Laboratories of Physiology in the Harvard Medical School

Received for publication February 23, 1935

The calorigenic action of fat has been explained as due to fat plethora (see Lusk, 1928). In diabetes, there is a high blood fat so that this fat plethora may be responsible for all or at least part of the increased metabolism of diabetes. The abundant ingestion of fat by the dog, as has been shown by Rubner (1902) and by Murlin and Lusk (1915), may produce a maximal increase in metabolism amounting to about 30 per cent of the basal level—a figure which is not far from the 40 per cent increase in metabolism which occurs as a result of diabetes in the cat (see Ring and Hampel, 1932). The questions of present interest were: Could fat ingestion by the normal cat increase metabolism by 40 per cent, and if so, would the blood fat reach a level similar to that found in diabetes? Would the respiratory quotient in normal animals after fat ingestion go below 0.75, which is common in diabetic cats?

It soon became apparent that cats were quite different from dogs in regard to the calorigenic effect of fat. Even when twice their daily caloric requirement was given in the form of fat, the metabolism was never increased more than 10 per cent, the respiratory quotient was scarcely lowered at all, and the blood fat was affected to only a minor degree. Arguing that a diminution in carbohydrate metabolism might be essential to increase fat utilization, I studied the effect of removal of the tail of the pancreas and a large part of the head. This procedure does increase the calorigenic action of fat.

With these partially depancreatized animals available, it seemed worth while to study the specific dynamic effect of carbohydrate as well.

METHOD, Measurement of metabolism. In measuring the calorigenic action of fat, one should have a continuous record of metabolism over a period of several hours. The apparatus was therefore arranged so that a small portion of the air leaving the box was continuously drawn into a bottle to occupy the space left by mercury which was flowing out at a constant rate. This air was later removed for analysis. Then by determining the rate of ventilation of the box, one could calculate the mean metabolism for the period. The ventilation was maintained at a rate

which caused the outgoing air to contain about 1.0 per cent carbon dioxide. The cat box rested on a platform supported by four springs. A lever attached to one end of this box recorded any movements of the animal. Usually these were so slight that they had a negligible effect on metabolism, but occasionally a period had to be discarded.

It was originally planned to measure the basal metabolism one day and the specific dynamic effect a few days later. Of course, slight differences in basal metabolism were expected on different days, but I felt that these would be of little importance in comparison with the large increase in metabolism to be expected after fat feeding. Experiments, however, showed that the effect of ingestion of large quantities of fat was very small in normal cats and usually within the range of variation of normal metabolism. Thus it became necessary to measure the basal metabolism and the specific dynamic effect on the same day. The final plan of an experiment was this. Cats were put into the respiration chamber at 9:00 a.m. and the compressed air was turned on. At 10:30, the cats had usually been quiet for at least half an hour and the outgoing air had reached a constant composition. Then three periods of one-half hour each were used to determine basal metabolism. After this the food, whose specific dynamic effect was to be studied, was given by stomach tube. The animal was allowed a half-hour in which to move about in a room and then returned to the box where the metabolism was measured during the second, third and fourth hours after the ingestion of the food. Previous studies had shown that a plateau of maximal metabolism was reached at this time. The above procedure has been modified by measuring the basal metabolism continuously for a period of five hours. The results, however, are constant throughout this period.

In the beginning, butter was used as the fat, but along with other refinements it seemed better to use a pure substance. Since oleic acid is easily obtained and will pass readily through a stomach tube, I continued the studies with this material. Of course, this is not a complete fat, but the results which I obtained were so similar to those found with butter that I believe the glycerine portion of the fat has very little to do with the specific dynamic effect. The oleic acid is for the most part supplied with glycerine and synthesized to neutral fat in the mucosa of the gastro-intestinal tract (see Munk, 1880; Munk and Rosenstein, 1891; Moore, 1903). The small amount of oleic acid which may reach the blood possibly upsets the acid-base equilibrium slightly, causing a blowing-off of carbon dioxide and thus a respiratory quotient which is too high. One would, however, expect that during the period of measurement the conditions within the body would be stable and the amount of fat (fatty acid) entering the blood would be equal to that leaving.

Partial pancreatectomy. If the tail of the pancreas and about three-

fourths of the head are removed, cats will usually digest food satisfactorily and will maintain weight without the use of insulin. Some will show a glycosuria, while others will not. The operation mentioned above is one spoken of as partial pancreatectomy.

RESULTS. In table 1 are given a few typical results of fat or fatty acid ingestion in normal cats. Each of the figures is the average of at least two measurements of metabolism and in most cases three or four. It seemed unnecessary to include the original figures because they checked so closely. It will be seen that, after the ingestion of oleic acid by normal cats, the metabolism scarcely changed except in cat 4, where the increase amounted to 10 per cent. The mean increase for the observations in this table is 2 per cent. Chambers and Lusk (1930) gave 10 grams of lard to dogs (about one-tenth the dose per kilogram which I gave cats), and obtained a mean increase of $3\frac{1}{2}$ per cent during the third, fourth and fifth hours after

TABLE 1
Metabolism of normal cats before and after fat feeding

CAT NUMBER	WEIGHT, KGM.	POSTABSORPTIVE		AFTER FAT (FATTY ACID) INGESTION		
		R. Q.	Calories per square meter per hour	Amount, grams	R. Q.	Calories per square meter per hour
1	3.15	0.75	28.3	40—oleic acid	0.74	28.6
1	3.20	0.76	29.9	40—butter	0.78	29.3
2	2.40	0.79	27.7	35—oleic acid	0.75	28.0
3	3.50	0.78	31.0	40—oleic acid	0.78	31.7
4	3.55	0.75	31.2	35—oleic acid	0.74	34.3

ingestion. Murlin and Lusk (1915) gave fat to dogs in amounts which more nearly correspond to mine and found an increase in metabolism amounting to as much as 30 per cent. Magnus-Levy (1894) observed during the seventh hour, after 210 grams of butter had been eaten by human subjects, a maximal increase of 9 to 14 per cent. Hiller, Linder, Lundsgaard and Van Slyke (1924) noted that 1 gram of fat per kgm. given to man caused a rise in basal metabolism which varied between 0 and 15.5 per cent, with the average at 8.5 per cent. All these observations lead one to believe that the dog and man show a greater calorigenic action of fat than does the cat.

If plethora is responsible for the calorigenic action of fat, there should be some correlation between blood fat and metabolism. Hiller, Linder, Lundsgaard and Van Slyke (1924) paralleled their calorigenic measurements with determinations of blood fatty acids. In their subjects, one showed a decrease in blood fatty acids, one an increase of 80 per cent, and four an increase between 1.3 and 29 per cent. Man and Gildea (1932)

gave 3.5 to 4 grams per kgm. to men and found the increase in fatty acids of the blood averaged 62 per cent. Bloor (1914) obtained an increase of about 100 per cent in the blood fat of three out of four of his dogs after a large fat meal. These latter results on man and the dog are in contrast to those obtained on 4 cats where the maximal increases were 8, 10, 22 and 23 per cent (unpublished), after giving about 10 grams of oleic acid per kgm. These observations harmonize with the fact that the metabolism in most normal cats is practically unchanged as a result of fat ingestion.

TABLE 2

Metabolism of partially depancreatized cats after fat ingestion

CAT NUMBER	WEIGHT, KGM.	POSTABSORPTIVE		AFTER OLEIC ACID INGESTION			PERCENTAGE INCREASE OR DECREASE IN METABOLISM
		R. Q.	Calories per square meter per hour	Amount, grams	R. Q.	Calories per square meter per hour	
2	3.25	0.79	27.6	40	0.77	32.7	18
5	3.65	0.77	31.0	40	0.76	37.4	21
5	3.75	0.78	31.0	40	0.74	38.0	23
5	4.00	0.81	32.9	30	0.80	36.3	10
5	4.00	0.79	35.5	35	0.83	34.6	-3
4	3.75	0.77	34.4	40	0.76	38.8	13
4	3.65	0.79	34.9	40	0.76	38.2	10
4	3.85	0.84	34.8	40	0.76	40.1	15
4	4.10	0.76	46.6	40	0.80	45.1	-3
6	3.80	0.71	30.0	35	0.74	34.9	16
6	4.25	0.77	32.0	20	0.75	37.2	16"
6	4.30	0.77	32.6	40	0.74	37.5	15
6	4.20	0.82	29.5	40	0.74	34.7	18
6	3.45	0.78	29.4	40	0.77	33.1	13
6	3.55	0.73	29.6	30	0.77	33.7	14
6	3.70	0.84	34.9	40	0.82	36.6	5
6	3.65	0.77	44.3	40	0.77	43.2	-2
7	4.15	0.80	24.5	40	0.78	27.9	14
7	3.65	0.70	37.1	40	0.72	35.9	-3

After partial removal of the pancreas, if the basal heat production stays about normal, the calorogenic action of fat is large—usually raising the metabolism by 15 per cent or more (see table 2). If, on the other hand, the basal metabolism is high, then fatty acid ingestion does not increase the metabolism further. In fact, the results are frequently lowered by 2 or 3 per cent. With intermediate basal metabolism the increases are frequently between these extremes.

The respiratory quotients are affected very little as a result of ingesting fatty acid. Five experiments showed an increase and thirteen a decrease. Of the five raised respiratory quotients, three were in animals having a high

metabolism and showing no specific dynamic effect. Had neutral fat been used instead of a fatty acid, the respiratory quotients might have shown a more constant and striking decrease after the ingestion. The results of the few experiments in which butter was fed were not noticeably different from those recorded in the tables.

Table 3 shows the specific dynamic effect of glucose in partially depancreatized cats. In most cases the caloric increase is not greater than 10 per cent. The respiratory quotients in all but cat 7 rose about 0.10, which is similar to results with the normal animals studied. This test would indicate that the capacity for burning sugar in most of these animals is not measurably decreased. In cat 7, where the respiratory quotient was low and was not raised by sugar, there was no specific dynamic effect. These results are included to show that in most cats studied the carbo-

TABLE 3
Metabolism of partially depancreatized cats after glucose

CAT NUMBER	WEIGHT, KGM.	POSTABSORPTIVE		AFTER GLUCOSE INGESTION			PERCENTAGE INCREASE OR DECREASE IN METABOLISM
		R. Q.	Calories per square meter per hour	Amount, grams	R. Q.	Calories per square meter per hour	
5	3.80	0.79	31.7	20	0.90	34.9	10
5	3.75	0.78	27.2	7	0.86	30.0	10
6	3.35	0.77	31.0	7	0.88	32.4	5
2	3.30	0.81	28.8	20	0.89	33.7	17
4	3.70	0.79	39.1	20	0.89	39.9	2
7	3.60	0.72	46.5	7	0.72	43.9	-6

hydrate metabolism was not markedly upset though the calorogenic action of fat was greatly increased.

DISCUSSION. The removal of a large part of the pancreas would be expected to decrease the pancreatic lipase entering the intestine and for this reason the fat absorption. This effect, however, is not large. Cruickshank (1915) showed that partially depancreatized dogs absorbed 72.12 per cent of the fat from a diet low in fat. Pratt (1916), also using dogs, stated: "When a bit of pancreas measuring 1 cm. in size was left attached to the open main duct, there was only a slight disturbance of absorption. A test made nearly six months after the operation and three weeks before the death of the animal showed that 75 per cent of the nitrogen and 66 per cent of the fat were absorbed." In the cats used, I have left more pancreas than Pratt, so that the derangement in digestion is perhaps very slight. Nevertheless, there is probably some decreased ability to utilize fat, and thus the calorogenic effect per unit amount of fat absorbed in the depan-

creatized animals is even greater than the comparison with normal cats would indicate.

The large calorogenic action of fat disappears whenever the operated animals chance to show a basal metabolism as high as that in diabetes. This fact, together with the greater calorogenic action of fat in partially depancreatized cats having normal basal metabolism, shows that there is a derangement of the fat catabolism of the diabetic animal.

The specific dynamic action of glucose also disappears with the high metabolism of diabetes—a condition to be expected when there is large excretion of sugar taking place even before glucose is given.

CONCLUSION

1. The maximal calorogenic effect of ingestion of large amounts of oleic acid by normal cats is insignificant (see table 1).

2. In partially depancreatized cats with normal basal metabolism ingestion of oleic acid produces a far greater effect than in normal animals (see table 2).

3. If the basal metabolism of partially depancreatized cats is high, the specific dynamic effect disappears (see table 2).

4. Most partially depancreatized cats show the same rise in respiratory quotient as that following glucose ingestion in normal animals (see table 3).

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DYNAMIC CONSIDERATIONS ON THE RELATION BETWEEN AORTIC AND VENTRICULAR PRESSURE CURVES

W. F. HAMILTON AND F. S. BRACKETT

From the Department of Physiology and Pharmacology, University of Georgia School of Medicine, Augusta, Georgia and from Research Associates, Inc., 3400 Nebraska Avenue, Washington, D. C.

Received for publication November 9, 1934

It is generally held that the work done by the heart can be roughly calculated by the formula

$$W = QR + \frac{MV^2}{2g}$$

The work (W) is the product of the volume of cardiac output (Q) times the blood pressure (R). To this is added the work necessary to accelerate the blood to the aortic velocity. This depends upon the mass of blood pumped (M) and the square of the velocity to which it is accelerated (V^2). In contrast to earlier preconceptions, Evans (1) shows clearly that the kinetic term $\frac{MV^2}{2g}$ is not to be ignored. Thus in a certain heart-lung preparation he shows that if the cardiac output is 0.67 liter per minute the kinetic fraction of the total cardiac work is 14 per cent; if the output is 1 liter per minute the kinetic fraction which increases geometrically is 25 per cent and if the output is increased to 1.7 liter per minute the work done in accelerating the blood reaches the remarkable figure of 50 per cent of the total work.

Evans calculated his results by taking mean velocity and pressure values. Katz (23) (working with the excised turtle heart) has shown that if these values are integrated the kinetic work is a still greater proportion of the total work. It should be pointed out, moreover, that 1.7 liter per minute is a rather small cardiac output for a normal resting dog and that figures well above these are to be found in the literature (2, 3, 4, 6) so that the percentages mentioned by Evans are not excessive.

Not only is the velocity factor recognized as playing an important rôle in the calculation of cardiac work, but it is also taken fully into account in discussions of the peripheral blood pressure. Thus the fact that the femoral pressure exceeds the carotid is shown to be due to the conversion of velocity into pressure energy (24, 25) and similar principles are used in discussions of the Korotkow sounds (26). Many other authors make use

of these concepts in discussing arterial pressure problems (cf. 27) but the above may suffice as examples.

An interesting paradox becomes evident when one contrasts this widely held point of view with the generally accepted picture of the relation between aortic and left ventricular pressure curves during cardiac systole. They are usually pictured parallel and at about the same height. This, however, leaves no room for the kinetic term which we have just discussed. The energy to accelerate the blood can only come from the intra-ventricular pressure and this quantity must rise above the aortic pressure whenever the aortic velocity rises above zero. Furthermore its excess above the aortic pressure must always be proportional to the amount of kinetic work the heart is doing.

Wiggers (5, 6, 7) and Piper (8), however, have observed pressure curves of this type and, since they occurred with high frequency in records that seemed technically sound, these authors were at one time inclined to regard them as expressing true pressure conditions. We hope here to substantiate this earlier view and to give a quantitative account of the meaning of this pressure difference. The more conventional relationship, however, appears in most treatises (5, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 27).

The difficulties in getting records where the ventricular pressure exceeds the aortic may be twofold.

Most important is the fact that the opening of the chest and other manipulations necessary to take intracardiac pressures in the usual manner may reduce the stroke volume to $\frac{1}{3}$ of its preoperative value with no appreciable change in blood pressure (21). The barbituric anesthetics have a similar effect (6), and when the dog's blood pressure reaches low values such as 120/80 (mean 95 mm. Hg), the stroke volume may be $\frac{1}{6}$ normal (21). The low stroke volume of course reduces the kinetic factor and the excess ventricular pressure is minimized. Furthermore, the most convenient place for entering the exposed ventricle is just below the aortic orifice (5). At this part of the ventricle the velocity is nearly as great as that in the aorta, and the energy which exists as pressure at the apex has been changed to energy of motion at this part of the ventricle. In some of the earlier work on unoperated animals (12, 13, 17) the heart was entered through the aorta or one of its branches and the cannula was stopped just below the aortic valves, that is, in a region of high velocity. Here again a fraction of the ventricular pressure has been changed to kinetic energy and the aortic and ventricular pressure curves should be parallel.

EXPERIMENTAL. The pressure curves which we present here belong to the same series as those used to illustrate a previous communication (4). They were recorded by means of a new hypodermic manometer (4) of high natural frequency whose cannula is an ordinary Luer needle.

It can be used in the unoperated animal with only a local anesthetic.

All the complications of surgical operation and shock are avoided and it is possible to take simultaneous records from the apex of the ventricle and from the proximal carotid artery in an animal which is completely normal and unanesthetized.

In figure 1 there is a series of intraventricular pressure curves taken from the apex and simultaneously with a series of carotid pressure curves. The ventricular curves exceed the carotid by varying amounts. This variation is caused either by variation in the stroke volume due to respiration or by respiratory variation in the position of the intraventricular needle.

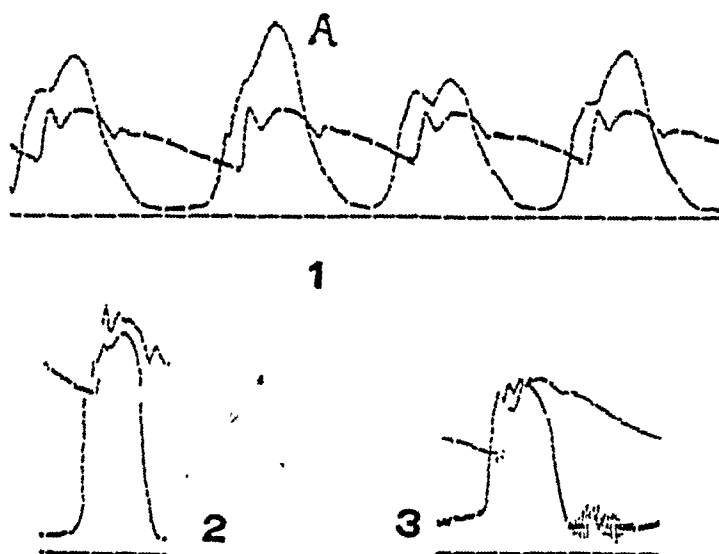


Fig. 1. Intracardiac and aortic pressure curves taken simultaneously from a normal unanesthetized and unoperated dog. Time $1/20$ second.

Fig. 2. Same taken from open chest preparation in poor condition. Ventricular pressure slightly higher than aortic. The two manometers differ somewhat in sensitivity.

Fig. 3. Same. Aortic regurgitation. Ventricular needle at mouth of aorta. Closed chest.

In figure 2 there is little or no excess ventricular pressure because the chest was opened and the animal in poor condition, though the blood pressure was high (adrenalin). The ventricular curve was taken from near the apex but the heart was not doing much kinetic work.

In figure 3 there is no excess ventricular pressure but the reason is entirely different from the preceding case. The pressures were recorded under local anesthetic and the chest was unopened. The animal was suffering from chronic experimental aortic regurgitation (2). The intraventricular needle was evidently in the base of the ventricle near the aortic orifice, because the turbulence which caused the diastolic murmurs impinged

directly upon the orifice of the needle and caused violent fluctuations of pressure within the manometer. With the intraventricular needle thus known to be in a region of high systolic velocity it is not surprising that the aortic and ventricular curves have summits that are essentially parallel.

Quantitative considerations. We were next interested to see whether a quantitative estimation of the aortic velocity, the volume output and the

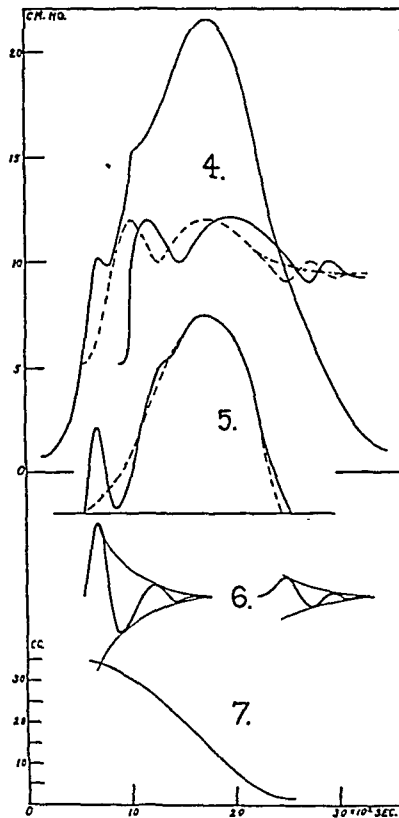


Fig. 4. Figure 1A. Plotted to one scale.

Fig. 5. Ventricular pressure minus aortic pressure.

Fig. 6. Transient pressure waves subtracted from figure 5 and giving the dotted curve in figure 5.

Fig. 7. Left ventricular cardiac blood volume during ejection, assuming $\frac{1}{2}$ sq. cm. area of aorta, 35 cc. total stroke volume.

ventricular emptying curve can be calculated from the values of simultaneous carotid and ventricular pressure curves.

In figure 4, the values of figure 1A have been plotted to scale. The full line curves show the pressure observed in the ventricle and carotid. Where one is dealing with a steady state and rigidly bound system, such a set of pressure data would enable one immediately to compute the velocity of the liquid.

Since, in our case, sufficient data are not available to permit a rigorous analysis, we shall make certain arbitrary assumptions. While these assumptions will leave the numerical evaluations subject to some uncertainty, it will be possible to draw conclusions from these calculations which will be definitely outside the range of uncertainty. If the contributions to the pressure differences due to time of pressure propagation and transient changes can be corrected for, we may then apply Bernouilli's principle to the evaluation of velocities.

The apex of the ventricle and the lumen of the aorta during ejection can be regarded for our purposes as parts of the same blood conducting tube. In the apex of the ventricle the velocity of flow can be regarded as zero, and that in the aorta can be regarded as maximal. Neglecting the slight losses due to friction, the total energy according to Bernouilli's principle is identical in the two parts of the system. Thus in the ventricular apex the pressure is high and the velocity low (zero) and in the aorta the pressure is lower and the velocity high. The relationship can be expressed by the formula

$$P_a + \frac{V_a^2}{2g} = P_v + \frac{V_v^2}{2g} \quad (\text{II})$$

Where P_a , V_a , P_v and V_v are pressures and velocities in the ventricle and aorta respectively. Letting $V_v = 0$ the formula for calculating aortic velocity becomes

$$V_a^2 = 2g (P_v - P_a) \quad (\text{III})$$

Two difficulties present themselves in applying these principles to the blood stream. First, since the walls of the system are semi-elastic, the pressure changes would be transmitted through the system with a velocity which depends upon the pressure values (even through energy loss to the walls is neglected).

Second, since there is a marked departure from steady state, momental considerations arise which somewhat modify the values obtained for the velocities. Such corrections, however would be small and cannot readily be evaluated.

It will be assumed that the pressure is propagated from one point of observation to the other in 2/100 of a second when the pressure observed is 100 mm.; second, that the propagation rate is linearly dependent upon the pressure (22). Thus, at 50 mm., 4/100 second is required. In other words, if the distance from one observation point to the other is 8 cm., the velocity of propagation at 100 mm. pressure is 4 meters per second.

The effect of the rapid rise in the propagation rate with pressure is of

great importance in connection with the transient effects which follow the initial pulse. At the initial valve opening, the first sharp pressure rise is transferred slowly down the aorta. However, by the time that the pressure has risen to twice the value, the pressure pulse is now traveling according to our assumption with twice the velocity. Thus the latter part of the pulse almost overtakes the first part, causing a much sharper pressure rise in the carotid than took place in the ventricle. The branching of the aorta creates a partial obstruction to the blood flow which is aggravated by the introduction of the cannula. This obstruction results in a reflection of the pressure pulse back to the ventricle, thus giving rise to a highly damped standing wave or transient.

The dotted curve shows the displaced values at the carotid as they appear, corrected for the time elapsed for pressure propagation.¹ The full line curve in figure 5 shows the difference in pressures when corrected for propagation time. It is fairly evident that a transient is superimposed on the general pressure change. If we assume a rather simple slow pressure change as indicated by the dotted curve, we have a residue of a typically damped disturbance which dies out just before the pressure difference reaches a maximum, as shown in figure 6.

While it is not possible to determine the extent of velocity disturbances which arise in connection with this transient, a computation of velocity (cf. 20) on the basis of the smoothed underlying pressure change cannot be greatly in error. When the pressure falls, due to the momentum of the blood leaving the heart, it falls below the value which would have been maintained if relatively steady flow conditions were realized. Furthermore, a reflection of the higher pressure condition from the carotid causes a subsequent pressure rise after the closing of the valve. Thus a transient and also highly damped standing wave is set up in the aorta which precedes the establishment of equilibrium.

In figure 4, the dash-dot curve has been drawn to indicate a probable value of the pressure corrected for this transient disturbance, the pressure values of the transient being evaluated as before in figure 5. Since the disturbance arises from a much less violent pressure change, the amplitude is much smaller than the initial transient but dies out in about the same length of time. The period of this disturbance, however, is less, due to the closure of the valve which shortens the path and creates a closed end tube. In evaluating the velocities of the blood leaving the ventricle, the higher values of pressure difference are more nearly correct than those of the steady state because of the persistence of the velocities due to momentum.

¹ We consider that in using a branch of the aorta we are measuring a pressure contour similar to that in the aorta itself. Perhaps it is identical in its major quantitative features, when the "transients" (see below) have been eliminated.

In this discussion we have attempted to bring out first the necessity of correcting for time of pressure propagation and second the influence of transients on the pressure changes observed. By a somewhat arbitrary procedure, a corrected pressure difference curve has been obtained which cannot be in error by more than a small percentage. By application of Bernoulli's principle (table 1, cols. 1, 2, 3), we obtain values for velocity of the blood stream leaving the heart (presented in columns 4, 5).² If we assume the cross area of the aorta to be 0.5 sq. cm., these data enable us to compute the volume of blood leaving the heart (column 6). Since this value for the volume agrees roughly with those obtained by other methods, the pressure differences exhibited must be of the right order of magnitude to bring about the purging of the heart.

Looking at the problem from an energy standpoint, work is done by the heart in expelling blood. A part of this work is spent in giving kinetic energy to the blood, i.e., energy due to its velocity; a part is spent in turbulence and transient disturbances; and a part is stored up in potential energy in the expanded arterial wall. From this standpoint, it is clear that the pressure differences arising in the heart must exceed those in the aorta in order to give the blood sufficient velocity to leave the heart in the allotted time. Since the volume of blood arrived at from our velocity considerations is the right order of magnitude, the pressures indicated by the manometers must not be regarded as anomalously high, but as absolutely essential to the mechanical process of purging the heart. If these computations are in error, they err on the side of requiring too small rather than too high a pressure difference. Since we have assumed that practically all except the first initial sharp rise in pressure is transformed into kinetic energy of the blood, we have placed a minimum requirement on the pressure difference. To whatever extent the pressure energy is lost in turbulence or potential energy of the aorta walls, to that extent the veloci-

² The velocity figures which we have given below are the inevitable conclusion from the pressure differences in the experimental record. If the cross area of the aorta is assumed to be 0.5 cm.² the total stroke volume comes out 35 odd cc. A larger cross area necessitates a proportionately larger stroke volume.

It should be noticed that the systoles immediately following and preceding the one analyzed in detail give evidence (see fig. 1) of much smaller velocities and hence of much smaller stroke volumes. For theoretical reasons the systole giving the greatest pressure difference is the one that should be taken as an example, the average systole probably gives rise to smaller velocities.

The fact that the femoral systolic pressure exceeds that in the brachial artery by 20-100 mm. Hg (25) might be taken to indicate that the velocity in the transverse aorta exceeds that in the iliac by figures of the same order as those which we attribute to the velocity in the aorta.

The fact that the blood velocity is greater than the pulse wave velocity is paradoxical. Of course the high velocities do not obtain out in the arterial tree—only in the aorta, and there only before the walls have taken up blood by elastic recoil.

ties must be reduced, thus corresponding to a smaller volume output for the heart.

It should perhaps be pointed out that all the above considerations are based upon the assumption that the normal movement of blood in the blood vessels is irritational, i.e., streamline. With this type of motion, pressure energy produces flow most economically, the work done being proportional to the square of the velocity imparted to the blood.

If, on the other hand, the stream of blood leaving the heart is turbulent,

TABLE 1

Aortic velocity and ventricular volume changes as calculated from the differences between aortic and ventricular pressure curves

TIME	$P_v - P_a$	$2g (P_v - P_a)$	AORTIC VELOCITY	AVERAGE DISTANCE TRAVELED BY PARTICLE OF AORTIC BLOOD	LEFT VENTRICULAR RESIDUE ON BASIS OF 35.93 CC. TOTAL EJECTION
sec.	cm. Hg	dynes/cm. ²	cm./sec.	cm.	cc.
0.06	0.11	2932.2	54.2	0.54	34.73
0.07	0.56	14927.4	122.2	1.22	34.12
0.08	1.21	32253.8	179.6	1.80	33.22
0.09	2.02	53845.1	232.0	2.32	32.06
0.10	2.92	77835.5	279.0	2.79	30.77
0.11	3.93	104758.1	323.7	3.24	29.05
0.12	5.10	135945.6	368.7	3.69	27.20
0.13	6.50	173264.0	416.2	4.16	25.12
0.14	7.60	202585.6	450.1	4.50	22.87
0.15	8.46	225509.8	474.9	4.75	20.50
0.16	9.11	242836.2	492.8	4.93	18.03
0.17	9.48	252698.9	502.7	5.03	15.57
0.18	9.40	250566.4	500.6	5.01	13.01
0.19	9.03	240603.7	490.5	4.91	10.56
0.20	8.31	221511.4	470.7	4.71	8.20
0.21	7.25	193256.0	439.6	4.40	6.00
0.22	5.40	143942.4	379.4	3.79	4.11
0.23	3.25	86632.0	294.3	2.94	2.64
0.24	1.57	41849.9	204.6	2.05	1.61
0.25	0.25	6664.0	81.6	0.82	1.20

there is an added loss which is also proportional to the velocity. When the blood leaves the heart quietly the aortic stream may not be turbulent, but whenever there are systolic murmurs or thrills, the aortic stream is probably turbulent. Under these conditions then the work which the heart must do to accelerate the blood is abnormally high.

It is to be hoped that the factors which we have attempted to bring out in a tentative and far from adequate analysis will emphasize the need for a further and far more rigid analysis of hemodynamic considerations in cardiac physiology and pathology.

SUMMARY

It is shown that the kinetic work done by the heart is accomplished because the intra-ventricular pressure markedly exceeds the aortic pressure during ejection.

Experimental curves of excess intra-ventricular pressure, when used to calculate the aortic velocity, the ventricular emptying curve, and the stroke volume, give results within reasonable limits.

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THE DIFFUSION OF POTASSIUM FROM RESTING SKELETAL MUSCLES FOLLOWING A REDUCTION IN THE BLOOD SUPPLY

A. M. BAETJER

From the Department of Physiology of the School of Hygiene and Public Health, Johns Hopkins University

Received for publication February 7, 1935

Potassium is normally maintained in skeletal muscle at a much higher concentration than in the body fluids. There are, however, many conditions under which a loss of potassium from the muscles may occur: injury and death of muscles (Horton, 1930, etc.), perfusion or immersion in solutions low in potassium (Ernst and Takacs, 1931, etc.), increase in acidity in the surrounding fluid over that in the muscle (Netter, 1928; Fenn and Cobb, 1934), possibly severe fatigue (Mitchell and Wilson, 1922), etc. A loss of irritability is usually supposed to accompany a loss of potassium from skeletal muscles.

The following experiments indicate that potassium may diffuse out of resting mammalian skeletal muscle under conditions other than those reported above, namely, when the blood supply to the muscles is temporarily reduced and that this may occur without apparently any marked loss in irritability or damage to the muscles.

METHODS AND RESULTS. Cats were used for these experiments, anesthetized with urethane. The blood supply to the right hind leg was reduced by three methods: 1, by loss of blood due to continuous bleeding; 2, by clamping the abdominal aorta; 3, by stimulation of the lower abdominal sympathetic chain, thus causing vasoconstriction in the muscles of the leg. The venous blood was collected over a measured period of time into 15 cc. graduated centrifuge tubes by cannulation of the femoral vein, heparin having been given intravenously to prevent clotting. After centrifugalization, the total volume and the approximate level of the red cells were read from the centrifuge tubes to give an approximate measure of the concentration of the blood. The rate of blood flow from the femoral vein was calculated in cubic centimeters per minute. Blood samples which showed any visible trace of hemolysis after centrifuging were discarded. The potassium content of the plasma was determined, after precipitation of the proteins, by the method of Kramer and Tisdall (1921), or Kerr's (1926) modification of this and in a few cases by the method of Shohl and Bennett

(1928). Variations in the concentration of potassium in successive blood samples of less than 6 per cent were considered insignificant.

The procedure used in the different methods of reducing the blood flow and the results obtained were as follows. After discarding the first few cubic centimeters which flowed from the vein after inserting the cannula, 10 to 13 cc. were collected as the normal sample. In four experiments successive samples of a similar amount were then collected continuously until respiration ceased, each sample being analysed for potassium. These experiments served as controls for the other experiments and for determining the effect of a reduction in the blood supply due merely to progressive loss of blood. When the venous blood was allowed to flow continuously without any other procedure, the potassium content remained constant

TABLE 1

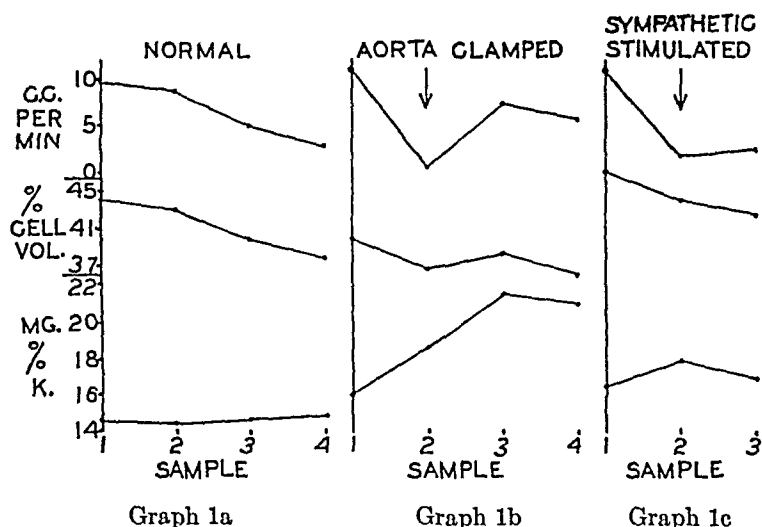
The effect of continuous bleeding on the potassium content of venous blood plasma
 Milligrams per cent potassium and percentage variation from first normal sample

SAMPLE 1, NORMAL	SAMPLE 2	SAMPLE 3	SAMPLE 4	SAMPLE 5	SAMPLE 6	SAMPLE 7	SAMPLE 8
17.82	17.98 +0.89%		18.97 +6.45%	22.02 +23.56%	34.30 +56.56%	Cat dead	
12.50	11.83 -5.36%	12.23 -2.16%	12.35 -1.20%	13.79 +10.32%	20.03 +60.24%	Cat dead	
13.16	13.94 +5.92%	12.49 -5.10%	12.94 -1.64%	12.86 -2.50%	13.21 +0.41%	13.57 +3.13%	15.39 +16.91%
13.92	13.82 -0.68%	13.26 -4.74%	13.78 -0.97%	14.98 +7.60%	Cat living		

within plus or minus six per cent of the first normal value until the last one or two samples just preceding the death of the animal when the potassium content rose rapidly, even reaching a value 60 per cent above normal. Table 1 gives the data for these four experiments, the absolute amount of potassium found in each successive sample and the percentage variation from the first normal sample being recorded. The average values for the amount of potassium, the rate of blood flow and the concentration of cells in the blood are plotted in graph 1a for the first four successive samples. The percentage of cells in the blood decreased progressively as did also the rate of flow.

In the second group of experiments a clamp was placed on the abdominal aorta during the collection of the second blood sample which varied from 5 to 23 minutes in the various experiments. The rate of blood flow through the hind leg was thus greatly reduced, the extent depending on the blood

pressure, the collateral circulation and the rate of dilution with tissue fluid. The potassium content of the plasma rose, usually markedly, in the blood collected during the period of clamping, varying from 2 to 42 per cent above normal with an average of 21.3 per cent, and continued to increase during the sample immediately following removal of the clamp (even if allowance is made for changes in the concentration of the blood) so that the potassium was then from 4 to 63 per cent above normal with an average of 44 per cent (table 2). The fourth sample showed a tendency in some cases to fall. During the clamping of the aorta, as shown by graph 1b (average of six experiments) the rate of flow and the percentage of cells in the blood decreased markedly while the potassium increased and during the following



Graphs showing the plasma potassium content (lower curves), concentration of blood (middle curves) and rate of blood flow (upper curves) in successive samples of femoral venous blood during continuous bleeding with normal conditions (graph 1a), with clamping of artery (graph 1b), and with sympathetic stimulation causing vasoconstriction (graph 1c). (Cats—resting conditions.)

sample the rate of flow and the percentage of cells returned toward normal while the potassium continued to increase. In the fourth sample all three values fell.

In the third group of experiments the blood supply was reduced by vasoconstriction brought about by stimulation of the sympathetic chain during the collection of the second blood sample, which lasted from 4 to 14 minutes in the different experiments. The potassium content of the venous blood collected during the sympathetic stimulation again showed an increase varying from 2 to 22 per cent over the first normal value with an average increase of 9.2 per cent for the 15 experiments (table 3). The potassium content of the plasma continued to rise in four experiments and

decreased slightly in eight experiments during the third sample. The rate of flow decreased with the vasoconstriction but showed a slight increase in

TABLE 2

The effect of clamping the abdominal aorta on the potassium content of the blood plasma from the femoral vein

Milligrams per cent potassium and percentage increase over first normal sample

SAMPLE 1, NORMAL	SAMPLE 2, CLAMPED	PER CENT INCREASE OVER NORMAL	SAMPLE 3, AFTER	PER CENT INCREASE OVER NORMAL	SAMPLE 4, AFTER
11.54	14.17	+22.8			
	16.43	+42.3	18.06	+56.5	
16.04	16.39	+2.2	16.69	+4.0	
13.83	17.71	+28.1	16.85	+21.8	20.70
18.26	23.93	+31.1	29.72	+62.8	30.04
16.50	20.16	+22.2	23.45	+42.1	21.20
21.20	23.82	+12.4			
12.33	14.27	+15.7	18.07	+46.5	16.42
13.11	16.95	+29.3	19.96	+52.2	18.99
14.29	15.47	+8.3			
	16.47	+15.3			
14.88	15.93	+7.2	18.80	+26.3	17.85

TABLE 3

The effect of sympathetic stimulation on the potassium content of the blood plasma from the femoral vein. Muscles at rest and intact

Milligrams per cent potassium

BEFORE SYMPATHETIC STIMULATION	DURING SYMPATHETIC STIMULATION	PER CENT INCREASE WITH SYMPATHETIC STIMULATION	AFTER SYMPATHETIC STIMULATION
15.95	17.23	+8.0	15.63
14.55	17.23	+18.4	17.86
19.18	23.53	+22.7	16.61
20.59	20.94	+1.7	20.78
21.30	22.36	+5.0	
23.78	25.20	+6.0	
22.72	25.56	+12.5	
14.40	15.37	+6.7	15.42
15.73	17.57	+11.6	18.73
17.93	18.47	+3.0	18.28
16.30	17.97	+10.3	25.23
(19.57)	21.21	+7.1	20.21
19.57	20.90	+6.8	20.23
18.91	19.89	+5.2	19.69
14.62	16.43	+12.4	15.34

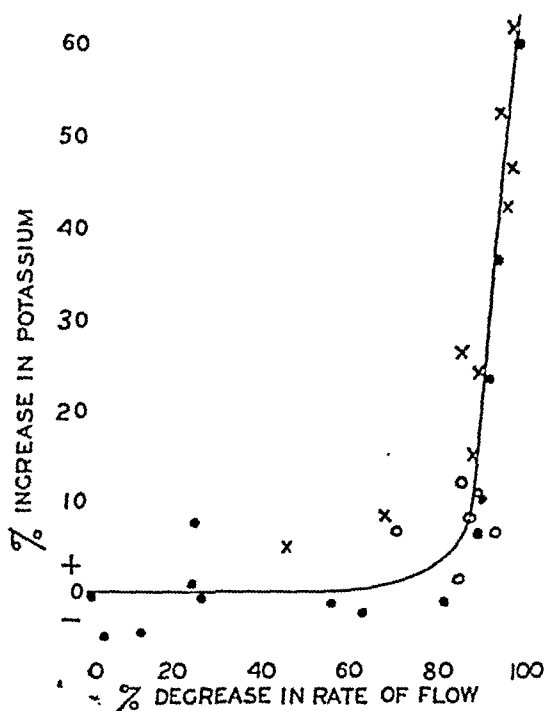
the third sample whereas the percentage of blood cells fell continuously as shown in graph 1c (average of four experiments).

In all of the above experiments the muscles were at rest. In five additional experiments blood samples were taken similar to this latter group but with the muscles contracting rhythmically throughout as a result of stimulation of the 6th and 7th anterior spinal roots. The potassium content of the venous blood plasma increased markedly (from 22 to 50 per cent above the normal value) in those blood samples collected during the period when the muscle reached the state of complete exhaustion no matter in which sample this occurred. This marked rise completely overshadowed any possible effect of sympathetic stimulation. As the blood pressure was already low due to the operative procedure, the loss of a very little blood resulted in a simultaneous slowing of the circulation and a rapid fall in the contractions. As the rate of flow was not measured in these experiments it is difficult to say how much of the increase in potassium could be attributed to the muscular exhaustion apart from that due to the reduction in the blood supply.

DISCUSSION. The above experiments show that under conditions of a reduced blood supply the potassium content of the venous blood returning from the limb of a cat contains a greater amount of potassium in the plasma than was present previous to the reduction in blood flow. When the percentage increase in the potassium is plotted against the percentage decrease in the rate of venous flow a most interesting relationship is seen, as shown in graph 2. As the rate of flow is decreased the potassium concentration remains fairly constant within the limits of -5 to $+8$ per cent, or possibly increases slightly, until the rate of flow is decreased about 80 per cent, which is equivalent to a rate of blood flow in the femoral vein of about two to three cubic centimeters per minute. From this point on, further reduction causes a marked rise in the concentration of potassium in the venous blood. The graph shows that the potassium increases approximately 25 per cent with 90 per cent reduction in rate of flow and 60 per cent with 98 per cent reduction in rate of flow. This latter value corresponds to about 0.3 cc. per minute. This relationship occurs no matter whether the blood flow is reduced by hemorrhage, vasoconstriction or clamping of the artery. In the clamping experiments since the potassium continued to increase in the blood sample collected immediately after the clamping period this latter amount probably gives a more correct value of the maximum potassium increase resulting from a reduction in blood flow and is therefore used in plotting graph 2.

As changes in the concentration of the blood occurred during the reduction in the rate of flow, all of the values both for the increase in potassium and the rate of flow were recalculated making allowance for the dilution assuming that the potassium content of the diluting fluid was equal to that in normal blood (see below). This correction did not, however, alter the general relationship.

It is assumed that this sudden increase in potassium in the venous blood with a reduction in the rate of circulation is due to the liberation of potassium from the skeletal muscle fibers. It is not due to concentration of the blood since dilution always occurred. Nor is there reason to believe that the source of the potassium is the extracellular tissue fluid which dilutes the blood when the supply is reduced since 1, the increase in potassium is not proportional to the amount of dilution; and 2, analyses of



Graph 2. The effect of a reduction in the rate of blood flow through the resting hind limb of cats on the concentration of potassium in the blood plasma from the femoral vein.

●—normal bleeding.

○—sympathetic stimulation.

x—artery clamped (allowance is made for the delayed increase in potassium obtained in the sample following the clamped period).

lymph show no greater potassium content than that in blood serum (Meyer-Bisch and Gunther, 1925) and the salt content of lymph and tissue fluid are identical according to Drinker and Field (1933). No increase in serum potassium is reported by Kerr (1926) in dogs 24 hours after severe hemorrhage when marked dilution had occurred. Although samples of arterial blood were not analysed it seems hardly likely that clamping the abdominal aorta or stimulating the lower abdominal sympathetic chain peripheral to its section could cause a rapid increase in the potassium of the general circulating blood. The increase in potassium after continuous bleeding, however, may be due to a rise of potassium in the general circu-

lation and may be derived from tissues other than skeletal muscle. There is also no reason to believe that the potassium is derived from the corpuscles of the blood under conditions of slowed circulation and its accompanying change in oxygen and carbon dioxide tension since Doisy and Eaton (1921) have shown that variations in the carbon dioxide tension of the blood do not cause any shift of potassium between cells and plasma.

The most plausible explanation for the increase of potassium in the blood would be to assume that the diluting fluid is derived, beyond a certain point, from the potassium-rich intracellular muscle fluid, or that the loss of tissue fluid from the muscle beyond a certain critical point is the direct factor in causing the potassium in the muscle to be liberated rather than the reduction in blood supply itself. However, when the absolute or percentage increase in potassium is plotted against the extent of dilution no such clear relationship is seen as shown in graph 2 for the reduction in blood flow.

The potassium is, therefore, apparently liberated from the muscle suddenly when the blood supply is reduced below a certain critical level and the question arises whether this corresponds to a sudden decrease in the irritability of the muscle. To test this, three experiments exactly similar to the most severe clamping experiments were performed, except that the sciatic nerve was stimulated at intervals before, during and after the clamping of the aorta and the resulting isotonic contractions of the *tibialis anticus* were recorded. No marked loss of irritability as thus measured occurred with a temporary reduction in the rate of flow of 95 per cent over a period of time longer even than in those clamping experiments showing the most marked rise in potassium. It must be noted, however, that the increase in blood potassium in these experiments must represent only a comparatively small loss from the muscles considering the high concentration of potassium in the muscles and the number of muscles involved. However, complete loss of irritability in contracting muscles, brought about by rapidly repeated contractions accompanied by a reduction in blood flow, was always associated with a rise in potassium.

Without knowing what other changes occur in the muscle and blood at the time when the potassium begins to diffuse rapidly from the muscle, and without further experiments to determine if a reduction in the oxygen tension of the blood, without a reduction in the rate of flow with its accompanying dilution, causes a similar increase in plasma potassium, it is futile to discuss the significance of the results described above.

These experiments were started originally in an attempt to show whether or not sympathetic stimulation causes a liberation of potassium, because potassium had been shown to have an effect on skeletal muscle somewhat similar to that obtained upon stimulation of the sympathetic chain (Baetjer, 1934). When it was found, as described above, that sympathetic stimulation caused the liberation of potassium into the blood, the question

arose as to whether this was a direct result of the sympathetic stimulation or a secondary result due to the accompanying vasoconstriction. These experiments on the effect of reducing the blood supply to skeletal muscle were therefore undertaken. It can be seen from graph 2 that there is no evidence that sympathetic stimulation as such liberates potassium, as no more potassium was obtained in these experiments than could be accounted for by the reduction in blood flow. If heart muscle loses potassium in the same manner as described above for skeletal muscle one wonders if the increase in potassium obtained by Howell and Duke (1908) upon stimulation of the vagus nerve was perhaps due to the accompanying cessation of the circulation in the heart.

CONCLUSIONS

1. When the blood supply through the leg muscles of a cat was reduced, either by hemorrhage, vasoconstriction, or temporary blocking of the arterial supply, the potassium content of the venous blood plasma showed no significant increase unless the rate of flow in the femoral vein was reduced to about eighty per cent of its normal value. Beginning at this point further reduction produced a rapid and marked increase in the plasma potassium. This is attributed to a loss of potassium from the muscles.

A temporary reduction in the blood supply such as led to this marked rise in plasma potassium did not cause any marked decrease in the irritability of resting skeletal muscle as measured by the height of occasional isotonic contractions.

2. Stimulation of the lower abdominal sympathetic chain caused an increase in the potassium of the venous blood plasma but this was no greater than could be accounted for by the reduction in blood flow due to the accompanying vasoconstriction.

The author is indebted to the following persons for their assistance in making some of the chemical analyses reported above: Dr. N. D. Kehar, Dr. L. Kajdi, and Mr. William Fusting.

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THE EFFECT OF POTASSIUM (AND CALCIUM) ON THE CONTRACTIONS OF MAMMALIAN SKELETAL MUSCLE

A. M. BAETJER

From the Department of Physiology of the School of Hygiene and Public Health, Johns Hopkins University

Received for publication February 7, 1935

A certain optimal concentration of potassium, equivalent to or slightly higher (Hegnauer, Fenn and Cobb, 1934) than that in Ringer's solution or blood is considered necessary for the normal irritability of skeletal muscle. An excess of potassium above this, according to the generally accepted view, exerts a depressant action on muscular excitability which may lead to complete paralysis. An excess of calcium chloride on the other hand is usually, although not always, reported to have a beneficial effect on skeletal muscle contractions in lower concentrations but a harmful effect in higher concentrations.

Practically all of the experiments on this subject reported in the literature have been made on frog muscles. The following experiments, which do not confirm these results, were made on mammalian skeletal muscles with intact circulation.

METHODS AND RESULTS. Cats under urethane anesthesia were used. Rhythmical break shocks from an induction coil at the rate of 120 per minute were applied to the 6th and 7th anterior roots, to the sciatic nerve or directly to the muscle, and the resulting isotonic contractions of the right tibialis anticus muscle were recorded. In some experiments blood pressure tracings were obtained from the carotid artery and in a few experiments plethysmograph records of the leg volume were also made. At intervals, while the muscle was contracting rhythmically, $1\frac{1}{2}$ to 2 cc. of the test solution were introduced, over a period of 5 to 15 seconds into the arterial blood stream supplying the right hind limb. This was accomplished through a cannula placed in the left common iliac artery close to the bifurcation of the aorta. For control two cubic centimeters of Ringer's solution with a potassium chloride content of 0.042 per cent were introduced at intervals so that any effect due to the addition of this small amount of fluid could be detected. All solutions were adjusted to a pH of approximately 7.4.

The results were uniform and definite. The introduction of two cubic centimeters of normal Ringer's solution was entirely without effect on the

height of the muscle contractions and on the blood pressure and produced only a very slight increase in leg volume (fig. 1a). When an excess of potassium chloride was added to the Ringer's solution so that the concentration of potassium chloride was 0.2 per cent or 0.4 per cent with or without a reduction in the sodium chloride content for isotonicity or when an isotonic 0.85 per cent potassium chloride solution was introduced, a marked temporary beneficial effect on the muscle resulted as indicated by a temporary increase in the height of the contractions (fig. 1b). These observations were repeated 38 times in a number of cats. A distinct improvement in the contractions occurred 32 times whereas no effect was ob-

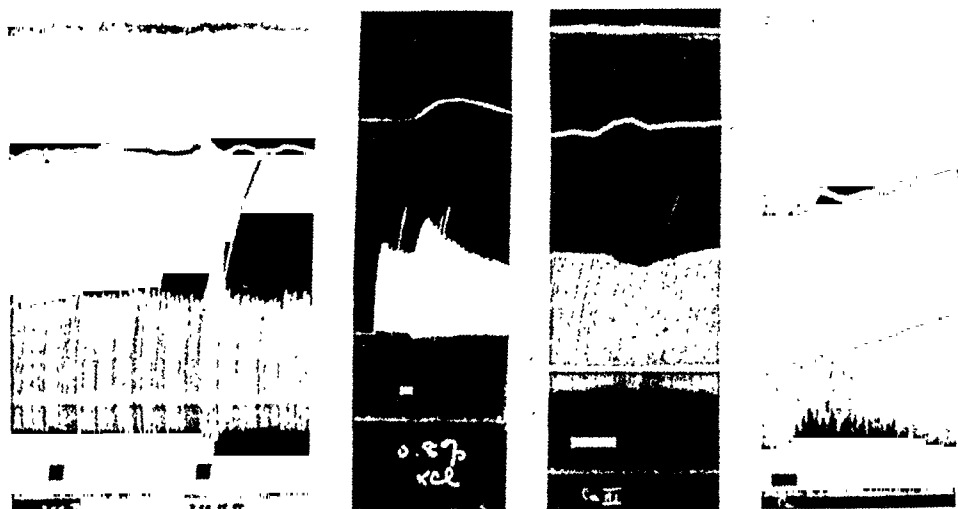


Fig. 1a

1b

Fig. 2

Fig. 3

Fig. 4

The effect of normal Ringer's solution, excess KCl and excess CaCl_2 on the leg volume (upper curve), blood pressure (second curve), and muscle contractions induced by stimulation of the motor nerve.

1a: Ringer's solution with 0.042 per cent KCl; 1b: Ringer's solution with 0.4 per cent KCl; 2: Isotonic 0.8 per cent KCl solution (curarized muscle stimulated directly); 3 and 4: Ringer's solution with 0.6 per cent CaCl_2 .

served 6 times. In these latter cases the contractions were falling rapidly in height and previous injections of potassium chloride had been made in rapid succession. The duration of the various phases of the response was as follows: latent period from beginning of injection of test fluid to beginning of rise in contractions, depending on the rate of injection, average 5 seconds, extremes 2 to 10 seconds; period of increasing contraction height, average 4 seconds, extremes 2 to 10 seconds; the maximal contraction height was maintained for only 2 or 3 contractions; period of decreasing contraction height, average 10 seconds, extremes 2 to 26 seconds. Usually the contractions returned to the level preceding the introduction of potas-

sium (20 cases). In 10 cases, however, the contractions although decreasing from the maximum remained at a level about 10 per cent higher than their original height showing a prolonged beneficial effect. In one experiment where 4 successive injections of potassium chloride were given the rise in the last two cases was followed by a sharp temporary fall in the contractions. This never occurred again although similar conditions frequently prevailed. The increase in contractions with successive injections of potassium chloride showed a tendency to become less but this was not always the case.

The extent of the increase in contraction height varied from 6 to 100 per cent with an average of 39 per cent. Too few experiments were made with the 0.2 per cent potassium chloride-Ringer's and the 0.85 per cent potassium chloride solutions to state definitely whether the increase in contractions varied with an increase in concentration or whether there was an optimum concentration, but the 0.2 per cent potassium chloride-Ringer's solution seemed to be less effective than the stronger concentrations. When the potassium chloride content of the test solution was 0.4 per cent, variations in the concentration of the sodium chloride between 0.9 per cent and 0.58 per cent did not affect the results.

The beneficial effect of potassium was obtained at all stages of the contraction curve, soon after the contractions were begun or later when the muscle was almost completely fatigued and the percentage increase did not show any relation to the height of the contractions before introducing the potassium chloride solution. This marked result of potassium on the contracting muscle was obtained whether the contractions were produced by stimulation of the motor roots, the sciatic or by direct stimulation of the muscle before and after poisoning with curare (fig. 2). It was also not influenced by poisoning of the vasoconstrictor fibers with ergotamine tartrate (Sandoz) and was independent of whether the sympathetic nerves were severed or intact, as would be expected. The improvement in the contractions was not due to an increase in the blood supply due to vasodilatation as the blood pressure usually remained unaltered or increased slightly and the plethysmograph records showed no alteration in leg volume which was not also obtained upon the injection of the Ringer's solution.

For comparison with the above effects of potassium on the contractions of mammalian skeletal muscle a few experiments on the effect of calcium were made in the same manner when the muscle was contracting as a result of stimulating the motor roots. In contrast to the increase in contraction height caused by an excess of potassium in the Ringer's solution, an excess of calcium produced a slight temporary fall in the contraction height. This occurred whenever the concentration of the calcium chloride in the Ringer's solution was 0.49 or 0.65 per cent (figs. 3 and 4),

usually when it was 0.33 per cent and occasionally when it was 0.16 per cent. Concentrations of calcium chloride below this appeared to be without effect. A corresponding reduction in the sodium chloride content was not made in the Ringer's solution when the calcium chloride was increased, because, as noted above, this appeared to be unimportant under the conditions of these experiments. The average decrease in the contraction height was 15 per cent, the fall in contractions began usually 8 seconds after the beginning of the injection and continued for about 10 seconds before the minimum was reached. The recovery was much slower averaging about 36 seconds before the contractions regained their original height. The accompanying blood-pressure tracings showed a slight increase occasionally, indicating vaso-constriction but no apparent changes in the leg volume occurred. The experiments were too few in number to warrant any other conclusions concerning the effects of calcium. They are cited only for comparison with the potassium experiments.

DISCUSSION. These experiments demonstrate that potassium in concentrations exceeding that in blood may have a temporary beneficial effect on contracting skeletal muscle, which is the reverse effect of that generally reported in the literature. This difference may possibly be due to the fact that cats were used in these experiments whereas frog muscles were used in previous experiments. That potassium may have reversible effects, however, even on frog muscle has been demonstrated by Lange (1924) for cell permeability, etc., and by Lapicque and Nattan-Larrier (1926) for muscle chronaxie. Another possible explanation of the effects here obtained may be found in the fact that the potassium solution was considerably diluted with the blood when injected into the circulation. The concentration of the potassium which reached the muscle must have been not over half that in the solution injected but even these concentrations have been reported to depress the excitability of frog muscle. It is possible, however, that the presence of other ions in the blood may alter the action of the potassium ions.

The effect of the potassium is apparently due to a direct action on the muscle itself rather than on the myoneural junction since the effect persists upon direct stimulation of curarized muscle. The explanation of this beneficial effect is impossible with our present lack of knowledge concerning the relation of potassium to skeletal muscle. However, if potassium passes into muscle cells as KOH or in exchange with H ions as suggested by Netter (1928) and Osterhout (1930), this would lead to an increased alkalinity within the cell. Fenn and Cobb (1934), however, found this to be true only to a limited extent.

An excess of calcium, on the contrary, appears to slightly depress the muscle contractions in cats temporarily. This may be due in part to a vasoconstrictor effect although this was not very evident. The reaction

is much slower and less intense in every respect than that produced by the potassium. These results seem to agree with those reported by Benda (1914) rather than with those of Toda (1930), Bouchaert and Belehradek (1927) and Gellhorn (1931, 1932).

CONCLUSIONS

1. The introduction of a small quantity of a solution containing 0.2 to 0.8 per cent potassium chloride into the arterial blood stream of cats produces a beneficial effect on the skeletal muscle as indicated by a marked temporary increase in the height of the contractions. As this result is obtained when the muscle is rhythmically stimulated directly after curarization as well as indirectly through the motor roots, the effect must be on the muscle fibers. This increase in the contractions is not due to an alteration in the blood supply.

2. A similar excess of calcium introduced into the blood stream produces a temporary diminution in the contraction height while solutions with lower concentrations of calcium are without effect.

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THE IMMEDIATE EFFECT OF SPINAL TRANSECTION ON THE CROSSED EXTENSION REFLEX

ALEXANDER FORBES, McKEEN CATTELL AND HALLOWELL DAVIS

From the Laboratories of Physiology of the Harvard Medical School

Received for publication March 6, 1935

Numerous researches have dealt with the effects of transecting the spinal cord upon the reflexes below the level of transection. The effects have generally been described as depression, and this depression has been commonly designated "spinal shock." In contrast with the commonly described depression is the fact that in the decerebrate animal (cat) the flexion reflex evoked by a single induction shock applied to a sensory nerve, whether recorded mechanically (Sherrington and Sowton, 1915) or electrically (Forbes, Cobb and Cattell, 1923), is greater after transection than before. This fact is interpreted by Sherrington and Sowton (1915) and by McCouch (1924) as due to release of the flexor center from the inhibition incidental to decerebrate rigidity (extensor tonus), the release being effected when the paths from the higher centers involved in maintaining extensor rigidity are interrupted by transection.

Forbes, Cobb and Cattell (1923) found that, whereas the immediate flexor response to the single shock was greater after spinal transection than before, the after-discharge was very much less, so that repeated stimuli evoked discrete twitches instead of cumulative contraction. They also found that, whereas before spinal transection the action currents in the motor nerve in a rapid succession of flexion reflexes (e.g., 4 or 5 a second) showed a marked decline in size from the initial value to a low "fatigue" level, after transection this decline had almost disappeared, successive responses holding up to almost the initial value. Endeavoring to relate these two effects of transection—decreased after-discharge (mechanically revealed) and the much smaller decline in the size of individual electric responses in the motor nerve—they suggested that the decline was due to an increasing "line-busy" effect as the discharge of motor nerve impulses increased in volume, and that the smaller decline after spinal transection was simply an aspect of the decrease in after-discharge following each stimulus, or cumulative discharge during a prolonged series of stimuli.

Later Gerard and Forbes (1928) found that the "line-busy" effect, when directly investigated in the decerebrate cat, proved to be too small and too transient to explain the marked and prolonged decline in the size of motor

nerve action currents in the flexion reflex evoked by a series of single afferent stimuli. A fatigue or "equilibration" effect was invoked to account for the major part of this progressive decline.

McCouch criticized the view that spinal shock, as related to the flexion reflex, could be regarded as a mere decrease in after-discharge, on the ground that the interruption of the cortico-spinal tracts in decerebration introduced the complicating element of extensor rigidity (as mentioned above) with its inhibition of flexor centers, and that, when this effect was removed by spinal transection, the resulting release from inhibition explained the increased size of response. He further emphasized the fact that before spinal transection an interval had elapsed since the initial interruption of the cortico-spinal tracts, which permitted recovery from the real cause of shock.

Forbes and Baird (1929) showed that the increase in the action currents of the flexion reflex, resulting from spinal transection, was essentially the same whether this was performed within half an hour of decerebration or at a later period. This observation did not answer McCouch's main contention that the inhibition of flexors involved in decerebrate rigidity might introduce a confusing factor in the interpretation of the effects of spinal transection on reflexes.

A similar examination of the effect of spinal transection on the crossed extension reflex should have a significant relation to the problems raised by the observations described above, for decerebrate rigidity is a state of sustained extensor excitation, and therefore would not involve an inhibitory effect on the extensor center, such as was invoked to explain the changes in the flexion reflex.

Since the crossed extension reflex is not accessible to the same sort of electrical examination that was used with the flexion reflex (see Forbes and Cattell, 1924), the contraction of an extensor muscle is the index which must be used. This may be supplemented by recording the action currents of the muscle.

METHOD. The essential features of the experiment were the application of single induction shocks or successive shocks at various frequencies to the sciatic nerve in one leg of the decerebrate animal and recording the contractions of an extensor muscle in the opposite hind leg, and rapid transection of the spinal cord without disturbing the set-up of stimulating or recording systems. Cats were used in all experiments.

Under deep ether anesthesia the spinal cord was exposed at the level of the last rib and a thread was passed under it to facilitate transection. It was then packed with moist cotton and the wound was closed. Next the animal was decerebrated at the level of the anterior colliculi. Ether was then withdrawn.

Stimulating electrodes were applied to the left sciatic nerve, severed

distal to the point of stimulation. A right extensor muscle was then prepared for mechanical, and sometimes also for electrical, registration. In nearly all the experiments the knee extensor, vasto-crureus, was used; in a few, the gastrocnemius. In the earlier experiments contraction was recorded isotonicly by leading a thread from the lower leg between knee and ankle (or from the foot, when the gastrocnemius was used) to a light lever, partially restrained by an elastic band, recording on a smoked drum. Usually the right sciatic nerve was severed in order to eliminate any possible confusing motions from the lower leg. In the later experiments, isometric recording was used. To this end, the system of drills and lever described by Forbes, Davis and Lambert (1930) was used, the psoas muscles and the saphenous, rectus femoris and obturator nerves being cut, in order to eliminate extraneous motions. With this apparatus, photographic recording is employed by means of the optical system of the string-galvanometer. The galvanometer was then used as a signal to record the time of stimulation, a small part of the stimulating current being led off from the secondary coil into the galvanometer circuit.

For stimulation, a balanced pair of coils was employed and single make and break shocks from it were delivered by a hand-operated mercury contact key designed to insure clean makes and breaks. For repetitive series of stimuli, sometimes the same key was operated by hand in such a manner as to produce from 4 to 8 stimuli per second. In other cases a rotary interrupter was introduced into the primary circuit, providing from 80 to 200 stimuli per second.

In a few experiments the electric response of the extensor muscle was recorded with a string-galvanometer, lead-off electrodes being of the Ag-Ag Cl type connected with the muscle by wicks impregnated with Ringer's solution and agar.

RESULTS. Seventeen experiments were performed, and incidental observations were made on six other preparations in which the spinal cord was transected for the study of other problems. Three of the animals died very soon after spinal transection, becoming moribund rapidly, apparently because of the hemorrhage resulting from the transection. None of these three showed the characteristic picture which was regularly found in almost all those that survived the transection in good condition. The observations made on these moribund preparations are of some interest and will be described later.

In 12 of the 14 preparations which remained in good condition, spinal transection regularly resulted in a change in the crossed extension reflex, similar to that already found in the flexion reflex, but much more pronounced. In the case of the flexion reflex, recorded electrically from the motor nerve, the response to a single stimulus was usually about twice as large after transection as before; in the case of the crossed extension reflex

usually no response to a single stimulus could be detected before transection, whereas after transection a fairly brisk contraction of brief duration was regularly evoked by a single shock. The most striking feature of the

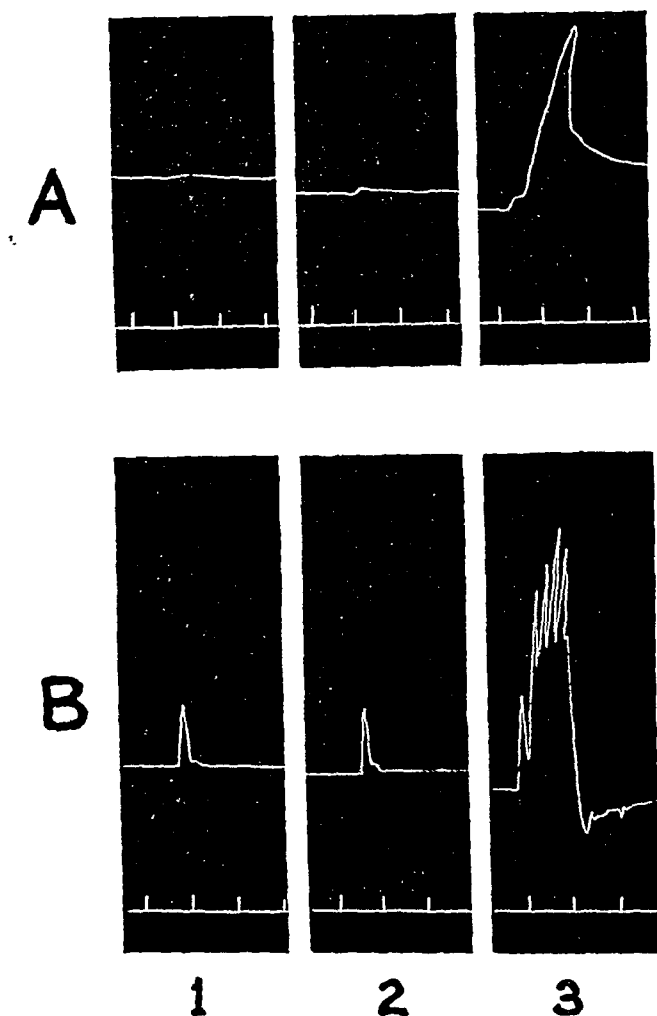


Fig. 1. Contractions of knee extensor, vasto-crureus, in decerebrate cat before and after spinal transection in thoracic region; recorded with approximately isotonic lever. Stimuli applied to opposite sciatic nerve. *A*, 10 minutes before spinal transection; *B*, 4 to 5 minutes after transection; 1 and 2, single break shocks (all the same strength); 3, repetitive make and break shocks by hand-operated key. Time in seconds shown below each record. November 19, 1923.

response after transection was its brevity, for, as in the case of the flexion reflex after transection, it resembled the twitch of a muscle stimulated through its motor nerve. Sometimes an extensor contraction was evoked by a single shock before transection, but in almost every case it was very

small. After transection the single-shock contraction was usually several times as large. Drum tracings showing this change in isotonic contraction are reproduced in figure 1.

The true values of contractile tension, and especially their time relations, are so much better revealed by the isometric lever that the character of the change caused by spinal transection is best shown in the experiments in which this method was used. Figure 2 shows the typical changes resulting from spinal transection, both in the case of single shocks and repetitive series of stimuli.

In contrast with the increased response to a single stimulus after spinal transection, we find a very great decrease in the response to a repetitive series of stimuli. Figure 2 shows this contrast between the effects of spinal transection on the single-shock response and that evoked by a "tetanizing" series, in two experiments. In both cases it will be seen that before transection the single shock evoked little or no contractile response, whereas the series of shocks produced the typical extensor reflex contraction of relatively long latency and gradually increasing contractile tension, indicative of "recruitment" (Liddell and Sherrington, 1923). After transection the single shock evoked a distinct contraction of brief duration, whereas the series of stimuli produced the same sharp initial twitch, followed by weakly sustained contraction at a level never higher (usually lower) than that attained in the initial contraction.

In figure 3 is shown a comparison of the responses to more slowly repeated stimuli before and after transection. These stimuli were delivered by hand, and the interval between them was sufficient to enable the contractile responses to appear as separate peaks after transection, whereas before transection, the stimuli being delivered at about the same frequency, the contraction, starting only after the second stimulus, continued to increase throughout the series. This same difference was also revealed in some of the isotonic records, e.g., figure 1. Examining the separate peaks in figure 3 *B* (after transection), it will be seen that the first contraction is much the largest, and that the size of successive contractions declines rapidly to a smaller value, suggesting fatigue ("equilibration"). Although in most respects the effects of spinal transection are similar in the two reflexes (flexor and extensor) one point of contrast should be noted. After transection the individual responses to the stimuli of a rapidly repeated series show well-sustained magnitude in the flexion reflex, but not in the extensor reflex. But this apparent difference largely disappears when we consider that in the extensor reflex before transection the individual increments of contractile tension, following successive stimuli, are almost lost in the smoothly progressing recruitment of the reflex. After transection the individual responses (fig. 3 *B*) appear as immediate

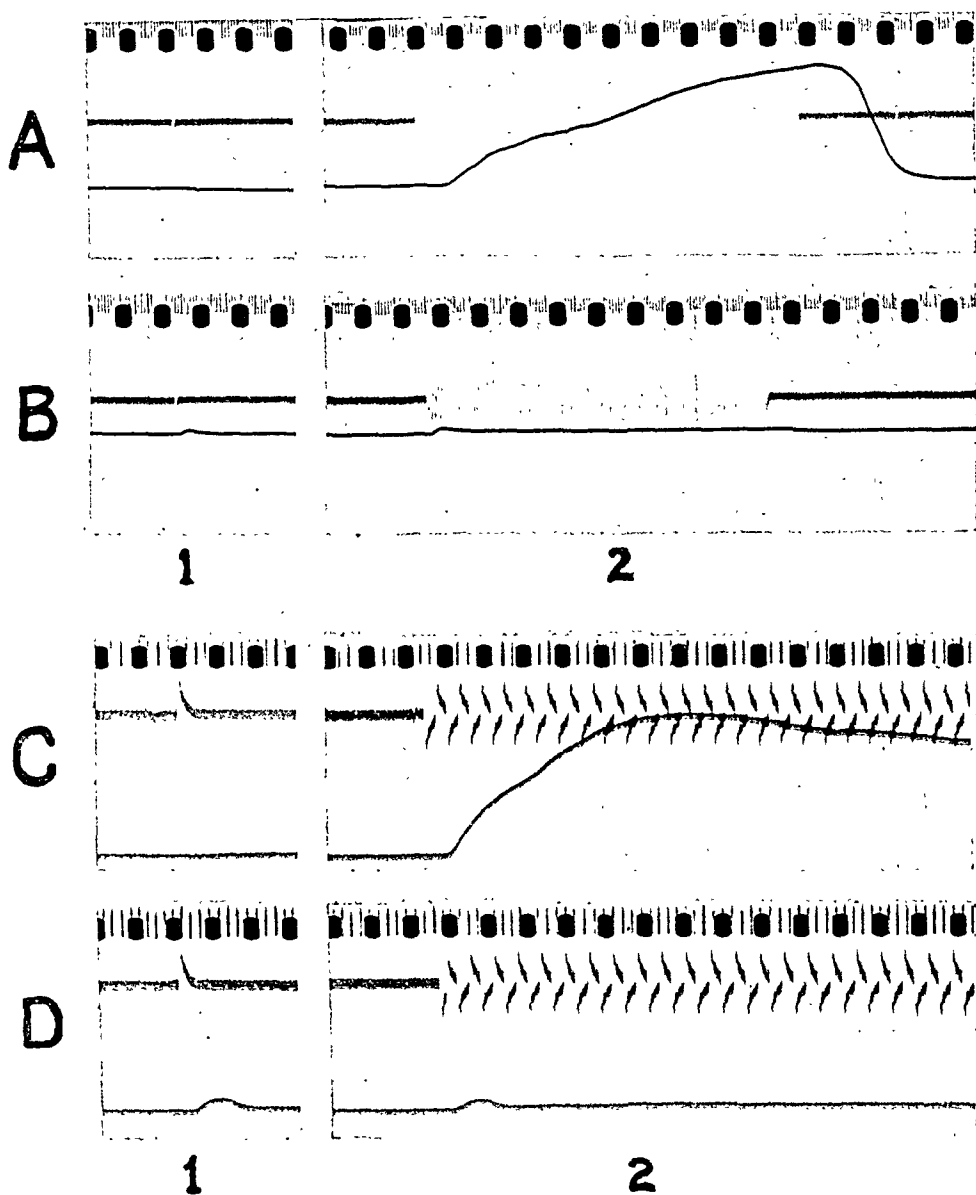


Fig. 2. Crossed extension reflexes recorded with isometric lever before and after spinal transection in two experiments.

In each record, upper line shows application of stimuli (part of induction shocks shunted through galvanometer); lower line shows contraction of vasto-crureus muscle. Time (0.01 sec.) shown above and between perforations at top of each record. *A* and *B*, May 10, 1928; *C* and *D*, December 19, 1934; *A* and *C*, before spinal transection; *B* and *D*, after transection (*B*, 12 min., *D*, 3 min. after).

In each record, 1 is single break shock; 2, tetanizing series of stimuli. Same strength of break shocks in all.

increments of tension as great as or greater than those correlated with the individual stimuli before transection.

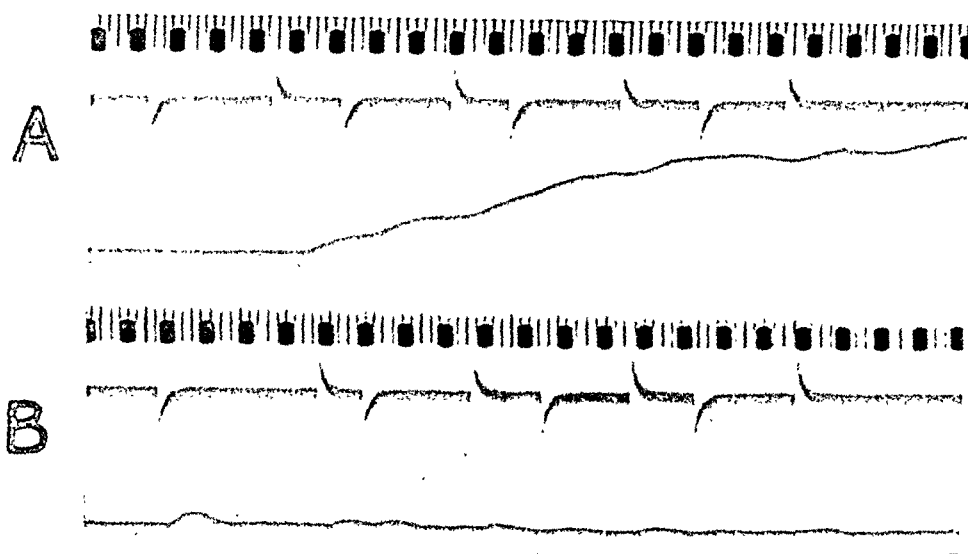


Fig. 3. Comparison of contractile responses in crossed extension reflex to slower series of stimuli, before and after spinal transection. Same arrangement as figure 2. A, 3 minutes before transection; B, 2 $\frac{1}{2}$ minutes after transection. December 19, 1934.

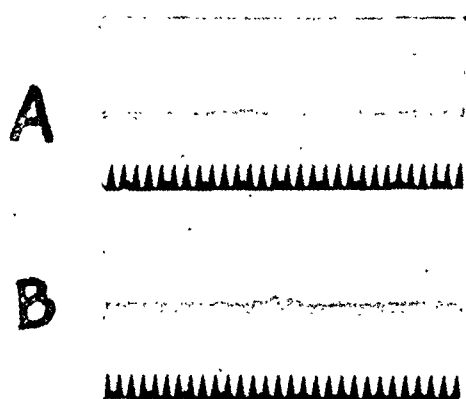


Fig. 4. Electric responses of vasto-crureus muscle in crossed extension reflex evoked by single shocks, before and after spinal transection. A, less than 3 minutes before transection; B, 10 minutes after transection.

Single break shocks of same strength in both. Time (0.01 sec.) shown below each record. March 30, 1923.

As noted above, electric responses of the extensor muscle were recorded in a few of our experiments. Examples of these are shown in figure 4. They give a comparison of the response to a single stimulus before and after

spinal transection, in the case of the vasto-crureus (knee extensor) muscle. In each case a small initial excursion of the string, caused by the electric artefact, shows the time of stimulation. In each case this is followed after about 30 milliseconds by the electric response of the muscle. In both cases the response seems to consist of two main peaks, suggesting a brief succession of repetitive discharges from the center. Clearly the response after transection is greater than before.

In the three animals which became rapidly moribund after spinal transection, although the flexion reflex persisted, and in one case even showed the characteristic increase, the crossed extension reflex completely disappeared. This suggests that the crossed extension reflex depends on a more vulnerable mechanism than the flexion reflex.

In two other experiments we failed to find the usual increase in the crossed extension reflex evoked by a single shock. In one of these, crossed extension was replaced on transection by crossed flexion, an unusual and anomalous reflex. In the other we found after transection the characteristic shortening of the latency of contraction, but a slight decrease in the size of contraction, instead of the usual increase. This observation was made on the gastrocnemius muscle, which in general showed the characteristic changes less distinctly than did the vasto-crureus. In spite of these exceptional cases, the regularity with which the changes described above appeared in a large majority of our experiments warrants the generalization that they are the usual consequences of spinal transection. This generalization is further supported by the above-mentioned incidental observations in six other preparations, all of which showed an increase in the crossed extension reflex as evoked by a single shock.

DISCUSSION. Our experiments show that in the decerebrate cat spinal transection produces immediate changes in the crossed extension reflex, closely similar to those previously reported in the case of the flexion reflex. In the extension reflex, they are even more marked than in the flexion reflex. These changes may be summarized as increase in the response to the single stimulus and decrease in the after-discharge as revealed in the cumulative contractile effect of a series of stimuli. This cumulative effect, which is the most salient feature of the crossed extension reflex in the decerebrate preparation, is entirely abolished by spinal transection.

As already mentioned, the increase in the flexion reflex upon spinal transection has been interpreted as a release from the inhibition incidental to the sustained excitation of extensor centers which causes the familiar condition of decerebrate rigidity. Clearly this explanation is not applicable to the similar change in the case of the crossed extension reflex, for in this case transection of the spinal cord interrupts a source of excitatory, and not inhibitory, activity coming from the higher centers. This leads to the inference that we are dealing with a general effect of spinal tran-

section upon the lower centers, common to the antagonistic flexor and extensor reflexes, rather than with a specific effect upon the flexor center.

Ruch and Watts (1934), in their comparison of the effects of spinal transection on reflexes above and below the plane of transection, note only a decrease in the extensor reflex in the hind limb. Since they apparently did not look for this reflex in response to single shocks, their results are not in conflict with ours, but rather exemplify the inferences that have resulted from the general use of repetitive stimuli.

We have mentioned above the inadequacy of the "line-busy" explanation to account for the effect of spinal transection on the flexion reflex. In the case of the crossed extension reflex, the increased response to the single stimulus might conceivably be explained as due to a decreased "line-busy" effect from the interruption of the tonic stream of impulses which maintain decerebrate rigidity. This effect might also explain the appearance of distinct twitches in response to a repeated series of stimuli in place of the slow cumulative contraction, but it cannot explain the marked decline in the size of the individual contractions of a series following the first, an effect which appears to depend on something in the nature of fatigue or equilibration.

Our experiments tend to reinforce the idea suggested in previous papers that much of the depression of reflexes called "spinal shock" in the older literature may have been due to the decreased cumulative effect of successive afferent volleys, since repetitive rather than single stimuli were generally used. Matthes and Ruch (1933) have described the crossed extension reflex evoked by a single afferent volley in the chronic spinal cat. They comment that this occurs despite the "depression of extensor reflexes in the spinal condition." Their observation accords with ours, while their comment reflects the prevailing view that depression is general. McCouch (1924) contends that apart from the change from the decerebrate to the spinal condition there is a depression of all reflexes below the level of transection following the first interruption of the cortico-spinal paths, and that, since that effect follows the initial decerebration, the result of a second transection lower down has nothing to do with the depression known as spinal shock. Clearly such an initial depression is not accessible to examination by the method employed in these experiments, since decerebration has already been performed before the observations are begun. Our experiments, therefore, give no answer to the question whether there is or is not such a generalized depression as has been commonly assumed. They do, however, suggest that some of the effects which have been grouped under the term "spinal shock" may have been due to the loss of the cumulative excitatory effect of repetitive afferent volleys, which our experiments show to occur not only in the case of the flexion reflex but even more strikingly in the case of the crossed extension reflex.

SUMMARY

1. Transection of the spinal cord above the lumbar enlargement in the decerebrate cat causes the following immediate changes in the crossed extension reflex: *a*, greatly increased muscular response to a single afferent volley (single induction shock); *b*, abolition of after-discharge and summation of central effects of successive volleys; *c*, decline in the size of individual responses of a series at frequencies which before transection caused cumulative contractile tension.

2. These changes are in the main similar to those previously reported in the case of the flexion reflex, but even more marked.

3. The explanation proposed for the flexion reflex, *viz.*, interruption of inhibitory impulses from higher centers, cannot apply to the extensor reflex, for these centers exert an excitatory effect on the extensor center. The similarity of the effects on these antagonistic centers suggests that the mechanism of the change is fundamentally the same in both.

4. The relation of these observations to theories of "spinal shock" is discussed.

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THE RELATION BETWEEN THE MOTOR AND SECRETORY FUNCTIONS OF THE HUMAN FASTING STOMACH¹

FRANCES A. HELLEBRANDT

From the Department of Physiology, University of Wisconsin

Received for publication March 4, 1935

In 1925 Lim, Ivy and McCarthy found that gastric distention provokes secretion in dogs and man. Since tension also gives rise to motility, this led to a consideration of a direct association of the muscular and secretory phases of gastric function. It was accordingly suggested that an enteric reflex secretion might be initiated by the mechanical stimulation of nerve endings as a result of compression. The hypothetical view was expressed that motility is probably invariably related to an increased secretion. Certain unpublished observations of Ivy were cited in confirmation of this supposition, for he had noted that the flow of juice is more abundant during hunger than quiescence. Carlson (1912) however had directly observed that during contraction the secretion of gastric juice was slow, mucin increased, free HCl decreased, and rapidity of secretion was accompanied by motor depression. In 1925 Hoelzel cited the nonuniformity of these views and commented that the abundant juice of Ivy was also higher in acidity. He reported new observations made on man. In an effort to keep intragastric conditions as nearly normal as possible, all but a small sample of the gastric contents were returned after aspiration and the tube was withdrawn, to be reswallowed again except when studies of secretory rate were in progress. The tube was then permitted to remain constantly within the stomach. Motility was subjectively gauged, or studied by the balloon method in the intervals between aspirations. Confirming Ivy, Hoelzel reported that acidity was in general higher during motility than quiescence. However, he found the contents of the fasting stomach smaller during periods of motor activity than quietude and the relative mucin content of the juice increased after strong hunger.

In 1934 we made concurrent inquiries into the secretory and motor phases of gastric function by the method of double intubation which we believe is superior to the one advocated by Hoelzel (Hellebrandt and Dimmitt). Studying the response to a small meal of gruel, we were impressed by the very striking parallelism between these two aspects of the behavior of the

¹ This work was made possible in part by a grant from the Wisconsin Alumni Research Foundation.

stomach. Protracted observations of the spontaneous fluctuations in the acidity of the contents of the fasting stomach (Hellebrandt, 1935) led us to extend our studies to the simultaneous investigation of the secretory and motor conduct of this organ when unstimulated by the presence of food. The results of this inquiry are presented in this paper.

METHODS. The fasting subject reported to the laboratory 14 to 20 hours after the last meal. A double or two single tubes were swallowed, one containing the regulation metal olive of the Rehfuß tube, the other, a small balloon of delicate condom rubber. The aspirating tube extended to the lower pole of the stomach. The balloon occupied a position about two inches cephalad. The subject assumed a position of repose in a deck or morris chair and remained asleep or quietly at rest during the period of observation which occupied from three to six consecutive hours. Changes in intragastric pressure were recorded by the use of a tambour and bromoform manometer. Samples of the gastric contents were removed quarterly. In general they were obtained with difficulty during periods of motor activity, and more freely during quiescence. As a rule, only three or four cubic centimeters could be secured and although aspirations were gentle and made with utmost care, occult blood in traces was commonly present in these samples, especially those withdrawn during hunger. Saliva was continuously removed by suction. As in our previous experiments, the observations were made upon healthy young adult women.

RESULTS. In our experience this experiment was difficult to perform, for the double tubes must be perfectly tolerated since nausea and retching vitiate the records. It was at times impossible to obtain samples adequate for titration. Occasionally a reflux of bright yellow bile distorted the acidity curves. During long periods of observation there was a tendency for the aspirating tube to be carried into the duodenum. Most frequently the observations were terminated because of increasingly strong evidence of trauma.

Over a period of 9 months, 10 successful experiments were completed on one subject. These included 20 hunger cycles during 14 of which satisfactory simultaneous acidity curves were gotten. Six additional observations were obtained on two other subjects. One series of aberrant findings was procured from a fourth. We were thoroughly familiar with this subject's ability to secrete hydrochloric acid. Upon one occasion the free and total acidity of her unstimulated stomach reached 79 and 117 respectively. Her response to histamine was normal. She swallowed and held the two tubes without apparent difficulty. Hunger was vigorous and typical. However, repeated aspirations after double intubation yielded consistently small samples which were colorless, thick and glairy. Within an hour they were so viscid that they were poured with difficulty from the tubes in which they were held. Free hydrochloric acid was uniformly absent.

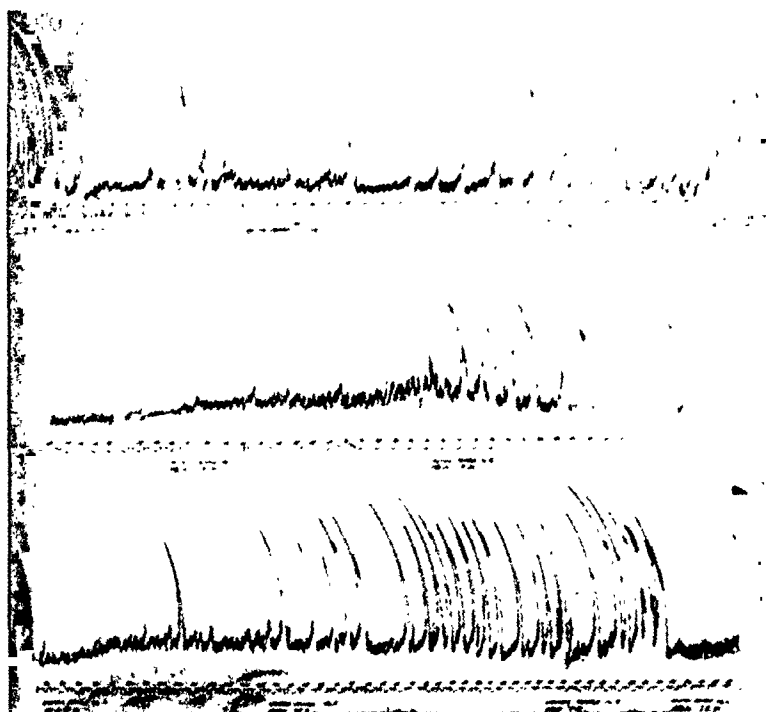


Fig. 1. Kymogram demonstrating the simultaneous secretory and motility data obtained by double intubation of the human fasting stomach. Scratch marks indicate the end of the period of aspiration.

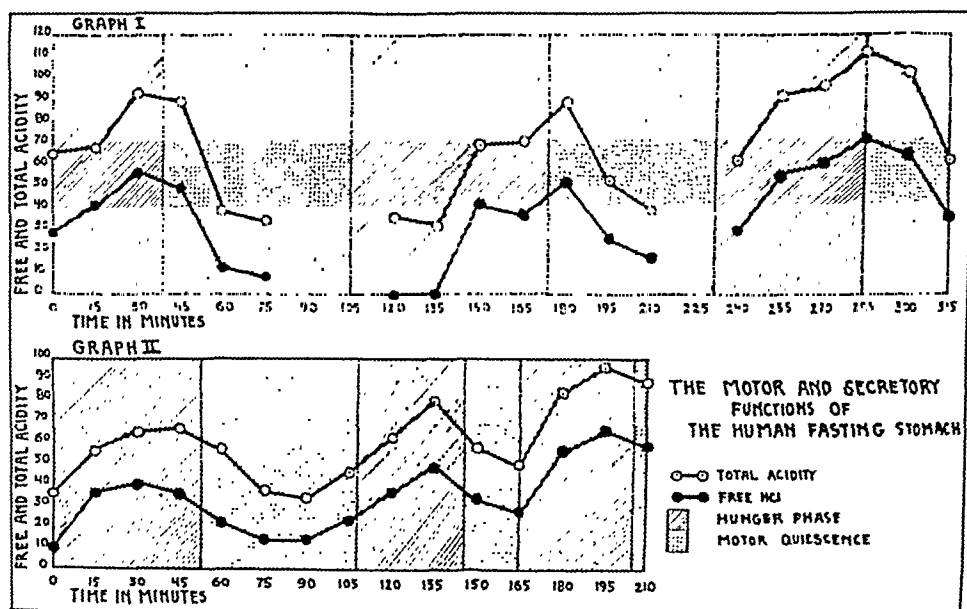


Fig. 2. Diagrams illustrating successive hunger cycles of the human fasting stomach and the fluctuations in the acidity of the gastric contents concurrently aspirated.

Without exception, every other observation showed the following four phenomena: 1, recurrent cycles of motility and quiescence; 2, recurrent fluctuations in acidity; 3, mounting acidity during hunger; 4, a prompt drop in acidity with the onset of quiescence. Figure 1 is a reproduction of a kymogram demonstrating recurrent hunger in subject R. E. B. The periods of relative quiescence are transitory but it is evident that during these intervals, gastric acidity is lower than during the phases of more vigorous motility. Graph I, figure 2, illustrates three successive hunger cycles recorded on subject S. L. H. and the concurrently observed fluctuations in the acidity of the gastric contents. Graph II represents three brief cycles obtained on F. A. H., who never developed the strong hunger and prolonged tetanus of the younger subjects, S. L. H. and R. E. B. However, the juice withdrawn was of relatively high acidity.

COMMENT. We believe that under adequately controlled experimental conditions an invariable relation may be demonstrated between the motor and secretory functions of the human fasting stomach, the acidity of the gastric contents increasing as the motility augments and subsiding with the onset of quiescence. Although normally recurrent cycles of hunger were observed in a subject with achlorhydria, we do not think this throws any light upon the mechanisms involved in the relation noted, because the secretory response was atypical, the excessive production of mucin obscuring the functional response of the acid secreting cells. We have no real evidence suggesting that the uniform relation between acidity and motility is or is not causal. Both phases of gastric function may be under the influence of some extra-gastric factor. We do not know if the two may be dissociated. It seems significant that the parallelism persists whether the stomach is in the fasting condition or under the functionally stimulating influence of food. From the practical viewpoint it seems imperative that cognizance be taken of motility in the evaluation of gastric fractional analyses.

CONCLUSION

Recurrent hunger cycles of the human fasting stomach are associated with fluctuations in the acidity of the gastric contents. These two phases of gastric function augment and subside in unison.

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THE RELATIONSHIP OF THE EXTRINSIC RENAL NERVES TO THE ORIGIN OF EXPERIMENTAL HYPERTENSION

IRVINE H. PAGE

From the Hospital of The Rockefeller Institute for Medical Research, New York, N. Y.

Received for publication March 8, 1935

It has been suggested that the elevation of blood pressure associated with either inflammatory or vascular disease of the kidneys may be due to nervous impulses originating in the kidneys and conducted by way of the extrinsic renal nerves to the vasomotor centers. This theory is now susceptible of experimental investigation. We have done this by severing the nerves of the renal pedicles before subjecting animals to two different procedures for the production of hypertension of renal origin.

Methods resulting in hypertension in dogs. The first method employed is that of Goldblatt, Lynch, Hanzal and Summerville (1934), an ingenious procedure for constricting the renal arteries.¹ A small silver screw clamp is placed on the main renal arteries and tightened sufficiently to impede slightly the blood flow.

The usual lumbar incision was made to expose the kidney. It was freed with its vessels from perirenal fat and peritoneum, the renal capsule being left intact. When denervation of the vessels was desired, they were cleaned of all visible nerves as far as the aorta and vena cava. In practice, it was found better to sever them close to the aorta where they are gathered in compact bundles and to strip them as they fan out toward the periphery. Nerves leading to the ureter were avoided. In some of the experiments the pedicle was then painted with phenol (3 per cent) dissolved in alcohol. A silver clamp was placed around the main artery and the screw tightened to compress the artery gently. Constricting the vessel more tightly results in thrombosis. The kidney was carefully replaced; the vessels must not be twisted. The wound was closed with gut reinforced by interrupted silk sutures. In some experiments clamps were applied to both renal arteries, and in others to the remaining artery after one kidney was removed.

In a second method, both kidneys were transplanted to a position directly under the skin of the flank, and the wounds allowed to heal. They

¹ Ligation of branches of the renal artery was performed by Pässler and Heineke (1905), Carrel (1909), Cash (1924), Mark and Giesendörfer (1930), to reduce renal tissue by necrosis of a portion of it. The method of Goldblatt, Lynch, Hanzal and Summerville produces renal ischemia instead.

were then treated with x-rays. If the remainder of the body is protected with lead plates the explanted kidneys can be irradiated without the use of deep x-rays. Treatment consisted of two erythema doses to each kidney at approximately four to six-week intervals. The details of this method, and the results of chemical studies of the blood, will shortly be reported in another communication.

Measurement of blood pressure. The van Leersum carotid loop method was found to be the most satisfactory procedure for the measurement of blood pressure in dogs. In this country the method has been described and modified by Cohn and Levy (1920) and more recently by Goldblatt² and his associates. It consists in sewing a length of the common carotid artery within a flap of skin so that it lies free on the neck of the animal.

Certain precautions have been found useful. If the skin is thin and loose the carotid loops are less likely to break. The skin flaps should be loose, since the tissues become edematous during the first few days after operation. Otherwise the sutures may burst or thrombi may form. Large pieces of absorbent cotton held in place by a gauze bandage have been found the most useful dressing, because they cling to the wound and prevent dirt from entering under the edges of the bandage, and because it is sufficiently soft to avoid constriction of the carotid loop. The dressing should be changed on the second or third post-operative day; afterward the wound should be treated with ointment such as butesin picrate until it is thoroughly healed.

Blood pressure measurements were made daily under environmental conditions as nearly similar as possible. The dogs were easily trained to sit quietly while the blood pressure was being measured. From ten to twenty readings were taken, and *that one accepted which seemed to represent* the most stable blood pressure level. The differences in pressure were not greater than 30 mm. of mercury.

Usually the operation on the carotid artery and unilateral nephrectomy were performed under amytal anesthesia (60 mgm. kilo given intraperitoneally). After complete healing had occurred (15–25 days), and the level of the blood pressure had been established (20–30 days), the renal nerves were severed and the clamp applied to the other renal artery.

Chemical methods employed in investigation of the blood. The hemoglobin was measured by the method of Van Slyke and Neill (1924), urea by the gasometric urease method (Van Slyke, 1927) plasma protein by the method of Howe (1921), and lipids by the gasometric method of Kirk, Page and Van Slyke (1934). Examinations of the blood were made after fasting for 24 hours. Water was, however, given freely.

² We are extremely grateful to Dr. Harry Goldblatt for many helpful suggestions concerning the application of their method, and for the silver clamps. He was also kind enough to allow us to undertake the problem, although he had had it on his program of future work.

RESULTS. In five dogs, about six weeks after performing unilateral nephrectomy, the remaining kidney was denervated and a clamp applied to the renal artery. Measurements showed that unilateral nephrectomy did not cause a significant rise in the level of the blood pressure, but constricting the renal artery did so shortly after recovery from anesthesia. A few days after operation the pressure in some cases measured 220 to 290 mm. Hg (figs. 1 and 2). If the renal nerves were left intact, a similar response occurred in two control animals. Denervation alone did not cause a significant elevation in blood pressure in two animals. Elevation of the blood pressure has already persisted for 240 days in animals apparently in good health. A trace of albumin was occasionally found in the

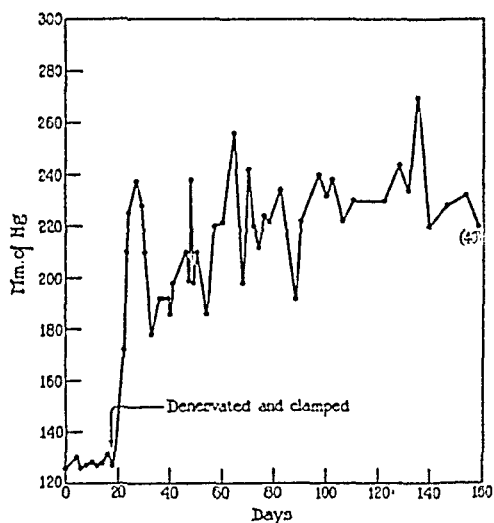


Fig. 1

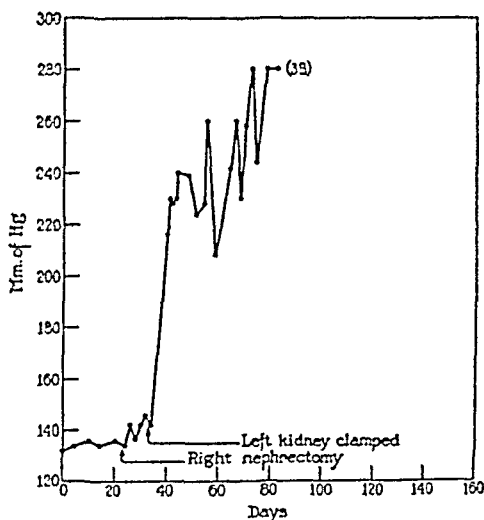


Fig. 2

Fig. 1. Dog. No. 40. Rise in arterial blood pressure following denervation and clamping of the renal pedicle.

Fig. 2. Dog. No. 38. Rise in arterial blood pressure following denervation and clamping the left kidney, after preliminary removal of the right kidney.

urine, as well as a few hyaline and granular casts. Hematuria was observed rarely, and there was little, if any, loss in power to concentrate urine. The arterioles in the fundus of the eyes appear to be constricted, but no hemorrhages or exudates have appeared. When both renal arteries are clamped, a rise in blood pressure similar to that just described has been observed.

No significant change in the chemical constituents of the blood has been detected, though a small but definite increase has occurred in the concentration of hemoglobin (table 1). Urea was either normal in amount or slightly increased, except when too great constriction of the renal artery caused renal insufficiency. Renal insufficiency, measured in terms of the urea content of the blood, was not a necessary nor usual accompaniment of arterial hypertension. The proteins in plasma were normal.

Lipids in the blood, including total fat, total and free cholesterol, phosphatide phosphorus, lipid amino nitrogen, and total lipid nitrogen, all were found to be normal in amount. This result also corresponds with clinical observations in the absence of signs of the nephrotic syndrome (Page, Kirk and Van Slyke, 1935). The only phenomena which may be unusual concern the ratio of free and ester cholesterol to total cholesterol, for free cholesterol increased at the expense of the esters during the course of the exper-

TABLE 1
Blood constituents of dogs with hypertension

NO.	HEMO- GLOBIN IN BLOOD IN TERMS OF O ₂ CAPACITY	UREA NITRO- GEN	PLAS- MA PRO- TEINS	PLASMA LIPIDS MGM. PER 100 CC.										AVERAGE BLOOD PRESSURE
				Total lipid carbon	Total lipid	Cholesterol			Lipid amino nitrogen	Total lipid nitrogen	Total lipid phosphorus	Total phos- phatides		
						Total	Free	Ester						
Unilateral nephrectomy. Remaining kidney denervated and clamped														
	<i>vol. per cent</i>	<i>mgm./ 100 cc.</i>	<i>mgm./ 100 cc.</i>											<i>mm. Hg</i>
Dog 38:														
Control.....	19.0	10.60	6.89	540	702	170.0	62.0	108.0	5.8	24.2	18.0	423		128
5 days after clamping..	17.2	10.37	7.06	445	578	138.5	48.8	89.7	3.0	17.6				230
19 days after clamping.	16.6	27.96	7.06	617	802	157.5	64.5	93.0	5.7	23.1	15.7	369		228
37 days after clamping.	24.48	79.35	6.00	677	880	183.8	93.3	90.5	8.6	32.8	16.7	382		>280
One kidney denervated and clamped														
Dog 40:														
86 days after clamping.	23.13	18.58	6.75	547	711	72.9	48.8	24.1	6.9	45.5	13.2	310		238
126 days after clamping.	22.40	13.87	5.49											
Unilateral nephrectomy. Remaining kidney denervated and clamped														
Dog 43:														
Control.....	18.66	13.6	7.25	663	861	194	69.8	124.2	5.8	38.1	18.2	428		136
12 days after clamping.	16.41	24.8	7.63	988	1284	184	111.3	72.7	6.7	35.9	22.9	538		218
51 days after clamping.	21.98	19.97	6.79	596	774					14.4	13.3	312		>280
91 days after clamping.	22.71	19.56	7.37	743	976	86	66.6	19.6	6.5	36.2	21.2	498		>280

Clinical observations. Dog 38. Animal died three days after the last blood examination. Post-mortem examination showed that a portion of the kidney was severely damaged.

Dog 43. This animal was quite sick following clamping of the renal artery. It is probable that the slightly elevated urea nitrogen and total fat were due to this.

iment. The liver may have been damaged, but no convincing proof has been found that this is so.

The results of the second method, irradiation of the transplanted kidneys, parallel those of the first. Bilateral renal denervation was performed on six animals, and six were employed for control purposes. Rises in blood pressure occurred in both groups after exposure to five to seven erythema doses of x-ray to each kidney. The level of the blood pressure usually

was not greater than 200 mm. of Hg, and was much more variable than that observed when the renal artery was clamped.

SUMMARY. Two methods have been employed in dogs, which have resulted in renal hypertension. The first, that of Goldblatt, Lynch, Hanzal and Summerville, consisted in constriction of the renal arteries by means of a silver clamp. The second consisted in transplantation of the kidneys to a position under the skin of the back, and, after healing of the wound had occurred, in irradiation with x-rays.

The first method produced sharp and sustained arterial hypertension regardless of whether the kidneys had been denervated. Except for rise in the concentration of hemoglobin in the blood, the constituents, including urea nitrogen, plasma protein, total fat, total cholesterol, lipid amino nitrogen, total lipid nitrogen and phosphatide (calculated from the lipid phosphorus) were not significantly altered. The amount of free cholesterol was increased somewhat at the expense of cholesterol ester. Increased blood urea concentration did not, as a rule, accompany the hypertension. Goldblatt and his associates (1934) have shown, by means of the urea clearance test, which is a more delicate measure of renal function, that hypertension and renal efficiency are independent of each other in dogs when clamps are applied to the renal arteries.

The results of the second method are parallel to those of the first, in that hypertension occurred as readily in animals in which bilateral renal denervation had been performed as in those which were the subjects of transplantation and irradiation alone. Hypertension was of shorter duration and not so severe as that produced by clamping the renal artery.

CONCLUSIONS

1. Since the production of arterial hypertension in dogs by constricting the renal arteries, or by irradiation of the kidneys with x-ray, is not affected by preliminary stripping of the renal pedicle of its extrinsic nerve supply, these nerves do not appear to participate in the genesis of renal hypertension.

2. Hypertension produced by constriction of the renal arteries does not result in significant changes in the proteins or lipids of the plasma. The hemoglobin content of the blood is slightly elevated. Renal efficiency, as measured by the content of urea in the blood, is not markedly altered, and bears no relationship to the height of the blood pressure, as Goldblatt, Lynch, Hanzal and Summerville have already found.

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AUGMENTATION OF THE PHYSIOLOGIC RESPONSE TO INSULIN

L. C. MAXWELL AND FRITZ BISCHOFF

From the Chemical Laboratory, Santa Barbara Cottage Hospital

Received for publication February 26, 1935

In spite of the voluminous research on insulin, successful attempts to slow down the resorption from parenteral depots to correspond more nearly to a physiologic rate of liberation have not been forthcoming.

Various protein precipitants and metallic salts have been found by Maxwell (1934) to markedly retard the rate of resorption of hypophyseal gonadotropic extracts. Since the physical and chemical characteristics (unpublished data) of the protein aggregate associated with the hypophyseal gonadotropic principle in some respects resembles that of the insulin complex, it appeared possible that compounds which were effective for the gonadotropic principle might also prove effective in retarding the resorption of insulin in the tissues. This paper deals chiefly with the action of basic ferric salts in prolonging insulin hypoglycemia.

METHODS. Adult rabbits and rats were used as test animals. The animals of each experiment were divided into two groups and fasted for twenty-four hours previous to dosage. One group of rats was used as controls and was given two units of insulin (Lilly) per kilogram of body-weight intramuscularly. The other group was dosed simultaneously with two units of the same insulin preparation to which had been added basic ferric chloride. After one week of rest the control and the insulin plus ferric chloride groups were reversed and the experiment repeated. This procedure controls any individual variation or change in insulin tolerance. The rabbits were subjected to a similar procedure with the exception of receiving one unit of insulin per kilogram body weight. Blood sugar values for the rats were determined by the Folin micro-method, and for the rabbits by the Schaffer-Hartmann method.

Insulin (Lilly) U 20, was added to a dilute aqueous solution of ferric chloride which had been previously neutralized to the point of precipitation. The pH of the solution of insulin after the addition of the basic ferric chloride ranged between 4.5 and 6. The mixture was diluted to contain two rabbit units of insulin and four milligrams of ferric chloride per cubic centimeter. The control animals were dosed with a similar dilution of insulin alone.

RESULTS. The mean average of the blood sugar values at various time intervals for the rats is shown in figure 1. It will be noted that the values for the control and the iron groups are identical at one and a half hours. At the third hour the mean of 34 ± 3 for the iron group (17 rats) is significantly less than the value of 46 ± 3 for the control group (17 rats). At the fifth hour the most striking effect is noted. The value for the controls at this time has risen to 69 ± 3.7 , while that for the basic iron group has remained between 30 and 35 ± 3.4 . At the eighth hour the blood sugar values of the controls have approached normal. At this time there was

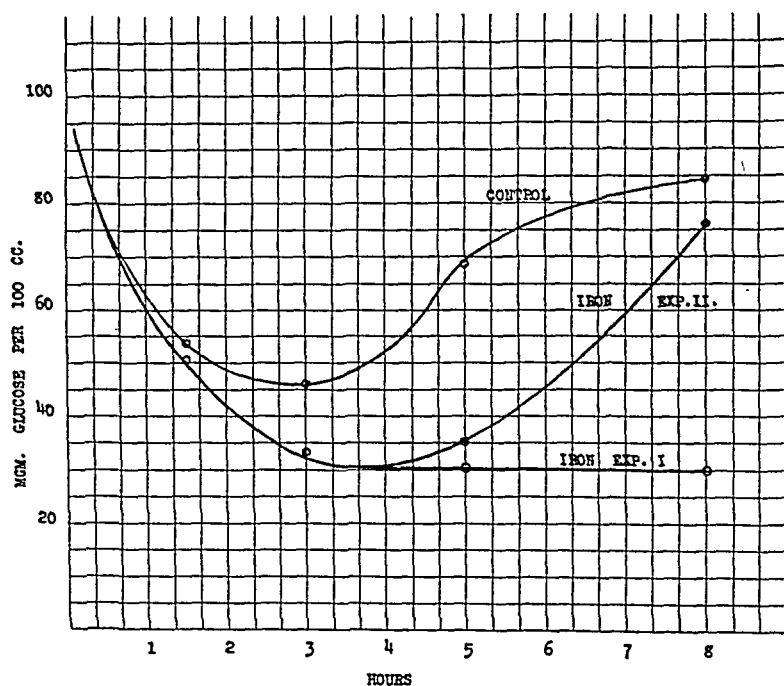


Fig. 1. The effect of addition of basic ferric chloride to insulin upon the blood sugar response of rats.

a difference in the response of the animals in the two experiments which comprise the iron group. In experiment I (6 rats) all the animals in the basic ferric chloride group were prostrated at the eighth hour, while in experiment II (11 rats) only one animal showed marked symptoms of hypoglycemia. This difference in response is illustrated by a division of the curve (fig. 1).

One group of three rats which had received the same dose of insulin to which had been added 4 mgm. of tannic acid per cubic centimeter previous to injection gave a blood sugar curve identical with that of the group receiving insulin plus basic ferric chloride. These results are not tabulated and

are mentioned only as evidence that the effects noted are due simply to a retardation in the rate of insulin resorption in the tissues.

The blood sugar data for the rabbits receiving insulin plus basic ferric chloride are essentially the same as for the rats. The data for the rabbit experiments are presented in table 1 in the order in which the experiments were performed.

Sahyun and Blatherwick (1928) in this laboratory found that after a pronounced hypoglycemia had been produced in rabbits, a ten-fold increase

TABLE 1

The effect of addition of basic ferric chloride to insulin upon the blood sugar response of rabbits

RABBIT	TREATMENT	BLOOD SUGAR VALUES IN MGM. PER CENT		
		3 hours	5 hours	8 hours
G	Control	76	130	106
	Iron	64	64	95
	Iron	50	40	45
W	Iron	62	58	92
	Control	62	112	116
	Control	30	92	103
7	Control	32	82	106
	Iron	24	20*	
6	Iron	56	96	106
	Control	64	98	
X	Control	56	88	105
	Iron	50	50	45
B	Iron	42	64	88
	Control	70	100	100

* Insulin convulsion.

in the dosage of insulin produced little change in the rate of fall of the blood sugar level during the first hour and a half. The effect noted was the prolongation of the hypoglycemia. Likewise the effect upon the blood sugar level produced by the addition of ferric chloride to insulin is not evident at one and a half hours, and is just significant at three hours after administration. It will be noted however that at the fifth hour the blood sugar of the group receiving insulin alone again approaches normal, while that of the group receiving insulin plus ferric chloride has not yet commenced to rise and only approaches the normal value at the eighth hour,

or in approximately half the cases even later. Sahyun and Blatherwick found that in order to produce differences in the blood sugar level of rabbits corresponding to those obtained by us at the fifth hour, it was necessary to increase the insulin dosage four- to ten-fold. This analogy indicates that when insulin is resorbed from the tissues at a rate corresponding to the more uniform physiologic liberation, the activity is greatly increased. Whether the decreased activity observed when insulin is rapidly liberated in the tissues is due to inactivation, or to an adrenalin defense mechanism, awaits investigation.

SUMMARY

The addition of basic ferric chloride to insulin augments the physiologic response as measured by blood sugar changes in both rabbits and rats.

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SEX-DRIVE IN RATS¹

JAMES ROLLIN SLONAKER

From the Department of Physiology, Stanford University, California

Received for publication January 22, 1935

In measuring the efficiency of rats by placing them in revolving cages one is confronted with the problem of the effect of adjacent rats on the animal under experimentation. If an effect on the activity of the experimental animal is produced by adjacent rats then additional variable factors would be introduced which might influence or nullify the results of the investigation. The object of this paper is to present certain observations which show that adjacent rats do exert an influence on spontaneous activity and must be considered in dealing with problems of efficiency.

It is well known that female rats during sexual life show marked rhythmic peaks of activity which are synchronous with the oestral period and that the isolated males show almost a uniform curve of activity—wholly devoid of rhythm (see fig. 1). We found, however, that the activity curves of male rats in cages near a female tended to follow the rhythm of the female activity. We concluded that there may have been at least two factors involved in modifying the usual male activity curve: 1, stimulation due to oestruation, known as *sex drive*; 2, increased activity of the female may have excited the rhythmicity in the male.

In order to throw some light on these possibilities males were subjected to three different laboratory conditions.

1. Eight males were isolated in a room with their cages arranged in a single row. The circumferences of these cages were 30 cm. apart. These were designated *At 1* to *At 8* consecutively. The activity curves for 40 days of the first two (*At 1* and *At 2*) in the row are shown in figure 1. During this period the ages were from 163 to 203 days. *At 1* showed a tendency toward a two-day rhythm. The greatest daily range of 3700 revolutions occurred between the fourth and fifth days. This range is much less than that usually shown by females (see fig. 2). The average daily run for the whole period was 8210 revolutions. The average daily run for the next

¹ This research has been assisted by The Department of Physiology, the National Research Council through the Committee for Research in Problems of Sex, the National Live Stock and Meat Board, Standard Brands, Inc., Albers Bros. Milling Company, Corn Products Sales Co., Swift & Company and the Golden State Company, Ltd.

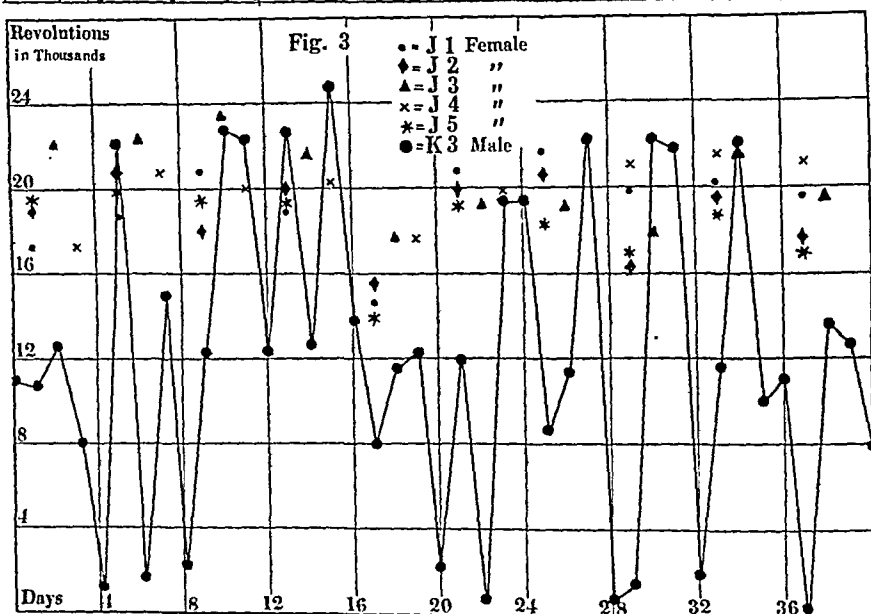
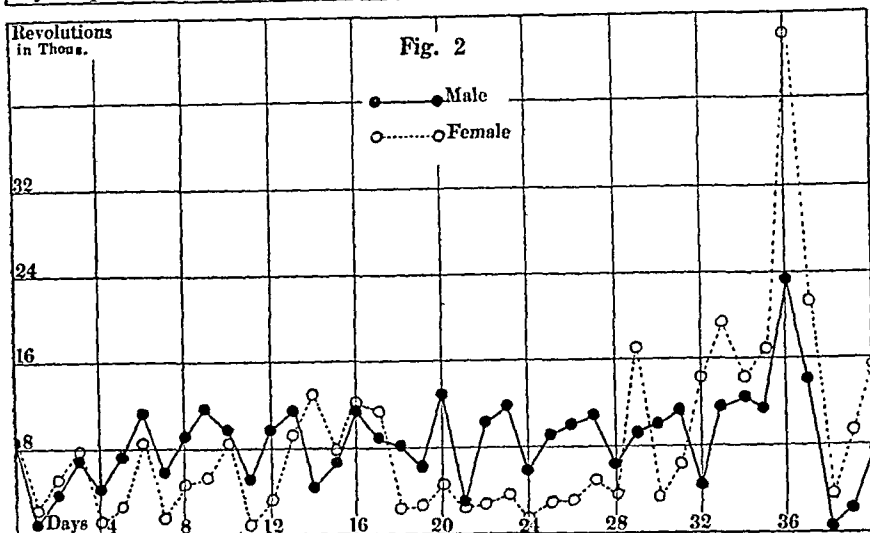
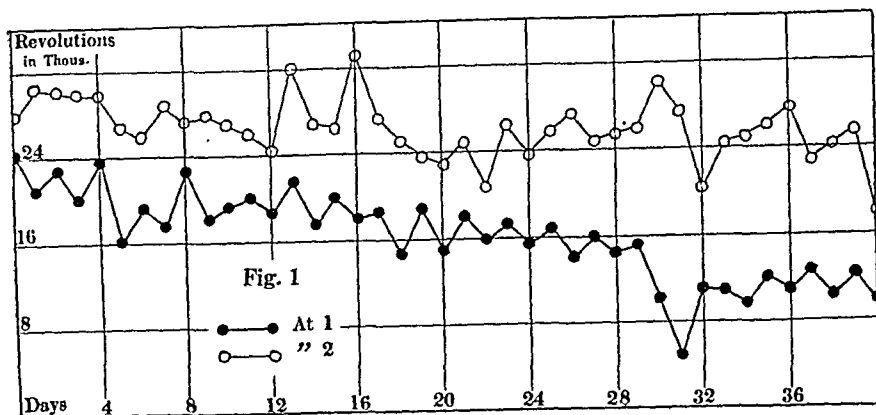


Fig. 1. Activity curves of males, *At 1* and *At 2*, which were adjacent to each other, but not near a female.

Fig. 2. Activity curves of male (solid lines) and female (dotted lines) of one of the groups of three, arranged in the order of male, female, male. The male curve represents the average run of the two males.

Fig. 3. Showing the activity curve of one male, *K3*, and the oestral peaks of closely adjacent females *J1*, *J2*, *J3*, *J4* and *J5*.

rat, *At 2*, for the same period was 13036 with a maximum daily range of 3800. During this same period the third rat (*At 3*) in the bank of cages averaged 10229 revolutions daily. The average daily run for the eight males in this group was 4723 revolutions. Figure 1 shows that *At 2* showed no tendency to a two-day, or any other rhythm. It also exhibits only a few cases of synchronous peaks of activity in the two curves. This number was approximately 15 per cent of the total possibilities. From this we conclude that increased activity of one male rat had little effect toward stimulating an increased activity on the part of adjacent males, the circumferences of whose cages were 30 cm. apart.

2. Three groups of three rats each were segregated in the colony room about three meters from any other animals. These groups were designated L, M and N. The order of the arrangement of the three rats in each group was identical. A female occupied the middle cage and a male on each side. The cages of each group were 15 cm. apart at their circumferences. Since each cage was 45 cm. in diameter the nearest either of the males could approach the female was approximately 30 cm. An increased activity of the males, synchronous with that of the oestral peaks of the female, would indicate that the female not only exerted a sex drive but that it could exert its influence over a distance of at least 30 cm. The activity curves of the two sexes of one group (M) for the same forty-day period are shown in figure 2. During this period the males were from 268 to 308 days of age and the female from 309 to 349 days. For the sake of simplicity and clearness the male curve represents the average daily run of the two males. By consulting this figure it is readily seen that the male curve approaches very closely that of a typical female curve and that the oestral peaks of the female are approximately in synchronous arrangement with the high peaks in the male curve. It is further seen that during the greatly reduced activity of the female from the 18th to the 29th day the mean activity of the male is not only maintained, but the peaks still respond chronologically to the slight oestral peaks of the female. The lack of complete accordance of the peaks in the two sexes may have been due to the sex drive stimulus having occurred earlier or later than the indicated female peak. We have no suggestion for the discrepancy shown on the 29th day. We thus see that not only was the activity of the male thrown into fairly definite rhythmic arrangement but that the daily fluctuations very greatly increased from a maximum of 3800 in figure 1 (rat *At 2*) to a maximum of approximately 14000 revolutions (rats M1 and M3). The average daily number of revolutions for the female during this period was 8078; for the males it was 8614. The average daily activity of these two males was 3891 revolutions greater than the daily average of the eight males in the former group. Since the rats of the former group were 100 days younger than M1 and M3, and were in the naturally more active phase of life, this difference in

average daily activity is more significant. This became more marked when the activity of these two groups at the same ages was compared. The average daily run then was 4691 greater for M1 and M2 than that of group 1. From these data we infer that the sex-drive stimulus was the main factor in bringing about these results and that the mere activity of the female had little or no effect. We thus see that the close proximity of a female induced both rhythmicity and greater activity of the adjacent males.

3. Five females, ages 139 to 179, and five males, ages 490 to 530, were used in the third group. One male, K2, died early in this period and its record was not used. The disposition of the animals in the third arrangement was as follows: A bank of cages containing the five females, J1 to J5, and a bank of cages containing the five males, K1 to K5, were placed with the cages end to end as close as their supports would permit. This brought the ends of the cages 9.5 cm. apart. K3 male could therefore approach that close to J3 female, but would be separated from J2 and J4 females by approximately 45 cm. and from J1 and J5 by 100 cm. All of these females had regular four-day periods except J4 which showed a six-day interval between the 23rd and 29th days. Three other cages containing males (K6, K7 and K8) of the same ages as the males K1 to K5 were placed at some distance from any female but in the same room. Fortunately the females came in heat on three successive days in the order of J1, J2 and J5 on the same day followed by J3 and J4. Then followed a day during which all were sexually passive. We thus had groups of three-day periods of sex-drive stimulus separated by a day of no stimulus. The first and third days this stimulus was exerted over a distance of from 45 to 100 cm., while on the second day only over 9.5 cm. Figure 3 illustrates the effect of these individual stimuli on the single male K3. For the sake of clearness only the oestral peaks of each of the females are indicated. They not only show the chronological order, but also indicate the number of revolutions each female ran that particular day. In the second arrangement previously described the sex-drive stimulus was present only one day in four while in this case it was operating three successive days in four. This figure shows that the approximation of a four-day rhythm exhibited by the males in figure 2 was completely eliminated in K3 and that the response of this male was due to one or more of the three possible stimuli in each four-day period. It is further noted that the peaks of activity and the daily fluctuations were greatly increased and that the days of great activity were usually followed by those of little activity. This suggests a state of lassitude or almost complete exhaustion during which no response was given to the stimulus. The maximum daily range was almost 22,000 revolutions. K3 was the most active of the four males. The average daily run of this male was 12209 revolutions and of the five females 10863

revolutions. The average daily run of all the males in this group was 4651 revolutions. The average daily revolutions of the three remote males, K6, K7 and K8, during this same period was 250. Since the age of this male during this period was from 490 to 530 days, an age during which the activity of males is relatively greatly reduced, a direct comparison of the activity curves in figures 1 and 2 does not present an accurate picture of the effect of sex drive on this animal (K3). The average daily runs of the males of figures 1 and 2 during the same ages as K3 were 3144 and 8240 respectively. A comparative summary of the activity of the different groups at the ages of the three periods used in figures 1, 2, and 3 is given in table 1. These averages show the effect of an increasing sex-drive stimulus on the activity of an adjacent male rat. In the later records it was further noted that between the ages of 550 and 650 days the response by marked

TABLE 1

Average daily revolutions of the groups at the ages given for figures 1, 2, and 3

AGE	GROUP At 1 to At 8				GROUP M			GROUP J AND K					GROUP K (REMOTE FROM FEMALES)	
	No.	Av.	At 1	At 2	Males		Female	Females (J)		Males (K)			No.	Av.
					No.	Av.		Av.	No.	Av.	K 3			
163 to 203	8	4723	8210	13036		*		5	14010	5	12591	14715	3	3198
268 to 308	8	4012	276	156	2	8614	8078	5	8478	5	6492	9633	3	508
490 to 530	8	3144	266	768	2	8240	9793	5	3988	4	4651	12209	3	250

* Not in revolving cages.

peaks of activity by the males became very sporadic and occurred more often as single responses which were separated by intervals of several days' duration. Occasionally groups of two or even three successive oestral-like peaks were elicited. With increasing age of the males the characteristic responses due to sex-drive stimulus finally ceased even though the adjacent females were still showing their regular oestral peaks of activity.

Figure 3 shows that, with only a few exceptions, the response of the male coincided with an oestration. A few instances will be cited. On the second day it reacted to female J3 and on the third day to J4. On the fourth day there was no sex-drive stimulus and the male showed little activity. On the fifth day J1, J2, and J5 were in heat and a burst of activity equivalent to almost 20 miles was elicited. We cannot state what part, if any, the oestration of J1 and of J5 had in this response. Females placed at different distances from males would determine the maximum

distance over which the sex-drive stimulus could operate. After this excessive run the male appeared to be so fatigued that it did not respond the following day to the closest female, J3. The seventh day it reacted to J4. On the 9th, 10th and 11th days a response was to all three of the possibilities. Obviously the vitality of the male did not permit a vigorous response to all of the stimuli. We attribute this lack of complete agreement of the high points of activity of the male to that of an oestral peak either to complete fatigue, or to oestrus having occurred a few hours before or after the reading of the revolution recorders. A later oestration of J3 would account for the male curve on the 27th, 31st, and 35th days. A few hours before or later would cause the high point of the male curve to appear twenty-four hours before or after the oestral peak. Hourly readings would be necessary to get an accurate representation of the results.

From the above we are led to conclude that when male rats are confined in revolving cages which are placed as close as 45 cm. from females in the prime of life, the spontaneous activity of the males will be modified and increased by a sex-drive stimulus. We have not determined over how much greater a distance this stimulus will cause a response. Our records show that these stimuli do not operate on the same sex. It follows, therefore, that in experiments on male rats, which involve spontaneous activity, the possible sex-drive stimulus of adjacent females must be taken into consideration.

SUMMARY

When male rats were confined in revolving cages to ascertain spontaneous activity the following results were observed:

1. When two cages, each containing a male rat, were closely adjacent, the increased or decreased activity of one did not seem to influence the activity of the other.

2. When a male and female were placed in separate cages so that the animals approached as near as 45 cm., the activity of the male was not only greatly increased, but was also changed into a marked rhythm similar to and approximately synchronous with that of the female oestral rhythm. The stimulus which caused this is known as sex drive.

3. With the addition of a greater number of females in close proximity to the male, his activity was still further increased and was no longer manifested in the characteristic oestral-like peaks elicited by the presence of a single female if oestration occurred on different days.

4. With increasing age of the male beyond 550 to 650 days the effect on the male of the sex-drive stimulus became less and less and finally ceased.

5. The activity or sex-drive stimulus of one female did not seem to affect the activity of an adjacent female.

THE PRESSURE CHANGES INDUCED IN THE VASCULAR SYSTEM AS THE RESULT OF COMPRESSION OF A LIMB, AND THEIR EFFECT ON THE INDIRECT MEASUREMENT OF LATERAL PRESSURES

H. C. BAZETT, L. B. LAPLACE AND J. C. SCOTT

From the Department of Physiology, University of Pennsylvania

Received for publication January 16, 1935

It has already been shown that though accurate estimates of end pressures may be made by the Riva Rocci method, particularly if the "formozsillatorisch" criteria of von Recklinghausen are used, yet during the process of compression and decompression considerable changes in directly recorded pressures may be observed, not only locally (Bazett and Laplace, 1933a, fig. 4), but also in the opposite femoral artery (*loc. cit.* fig. 3). In view of the fact that the criteria adopted as indications of lateral pressures (Bazett and Laplace, 1933b) were not definitely established as valid, further work to compare direct and indirectly measured pressures was undertaken, and this has shown that some of the pressure changes previously attributed by us to reflex adjustments have in reality a simpler mechanical causation. In this paper, consequently, data will be presented to demonstrate the local changes in pressure that are produced by compression, and the effect of these on the accuracy of criteria for measurement of lateral pressures will also be discussed.

The previous work of Erlanger and Hooker (1904), of Merke and Müller (1925), and of von Recklinghausen (1930) as well as the data reported by ourselves (1933a) makes it quite certain that the differences between end and lateral systolic pressures are considerable (10 to 33 mm.); consequently, in measurement of pulse pressure by the Riva Rocci method, the relationship of any criterion used to one or other of these pressures should be determined, since the whole principle of the Riva Rocci method for systolic pressure involves obstruction of the stream. Considerable differences of opinion exist as to the magnitude of the error involved. Maltby and Wiggers (1932) as the result of compression of a vessel in a clamp concluded that "the experimental evidence does not favor the view that systolic pressure readings obtained by clinically applicable criteria are necessarily too high"; on the other hand, Frank and Wezler (1931) considered that the reflection of waves may cause errors of 30 per cent or more in estimations of pulse pressure.

The problem, therefore, was to determine the effects produced on the actual pressures by the process of compression, and what criteria, if any, indicated the pressures existent in the system before interference by compression. This problem was attacked from three standpoints: *a*, experiments were conducted with a circulation schema essentially of the type described by Erlanger and Hooker (1904); in this the segment compressed was varied in type and size and the actual pressures were estimated by two optical manometers (Wiggers' pattern) inserted in the system on either side of the compressed segment; optical oscillograms of the pulsations of the compressed segment were also obtained; *b*, experiments similar to those with the schema were conducted with the compression chamber inserted in the thigh of a dog, so that the pulsatile changes were generated by the heart and retained their normal pulse form; *c*, the criteria demonstrable by oscillatory methods were compared in man with those obtained by auscultation on the same or opposite arm.

METHODS. *a. Experiments with a schema.* The schema is shown in figure 1; it resembles that described by Bazett (1924). The segment compressed in the glass compression chamber consisted of tubing of varying size and thickness, or of a section of dog's carotid of somewhat smaller bore than the smallest rubber tubing. The optical oscillogram was of the type described previously (Bazett and Laplace, 1933a). Corrections were made for any incomplete pressure equilibrium indicated by a shift in the oscillogram zero. The changes in the tube during compression were also watched through the glass and critical changes were signalled.

b. Experiments on animals. Dogs were used and were anesthetised as described in the previous paper (Bazett and Laplace, 1933a).

A chamber of the same type as that used in the schema was inserted in the thigh, with the manometers arranged as in the schema experiment. The femoral artery was dissected from the groin to the knee, and its lateral branches were tied. A section some 6 cm. in length was removed and was inserted into the chamber, and the upper end of the whole compression system was tied into the central end of the femoral artery. The lower end of the excised artery in the chamber was connected to the lower end of the main artery by a glass cannula, short length of heavy rubber tubing and the manometer. Heparin (20 mgm. per kilo) was injected intravenously to prevent clotting. In such experiments good oscillogram records could be obtained but the local circulation was abnormal since most of the branches of the femoral were tied, and the normal velocities in the vessel must have been considerably reduced.

In other animal experiments the procedure was consequently modified. A single Wiggers manometer with a simple cannula was inserted into the profunda femoris in such a way that its orifice was at right angles to the stream in the femoral artery. The peripheral pulse was recorded from a

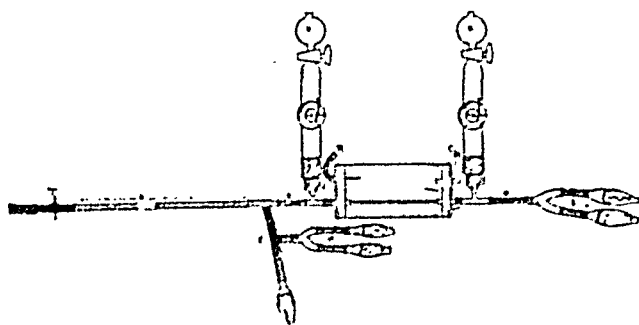
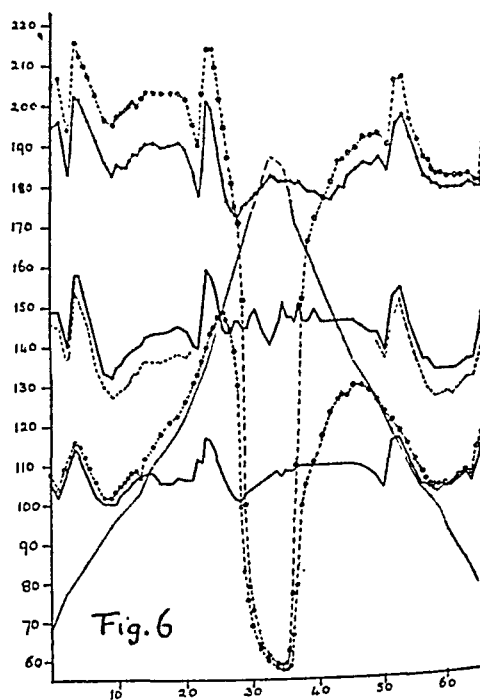
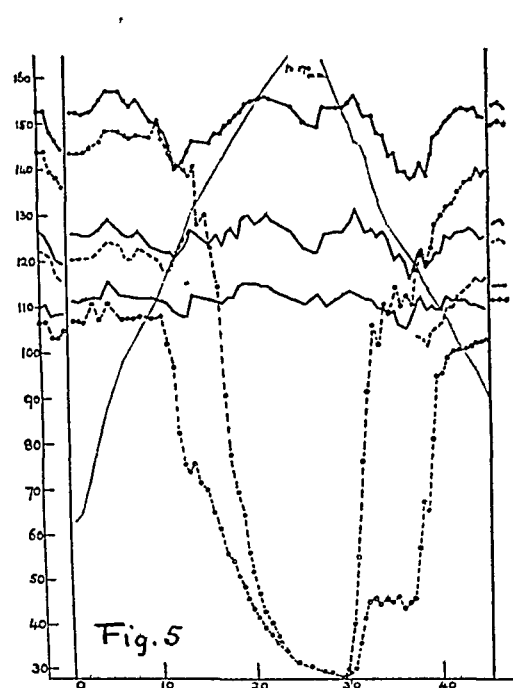
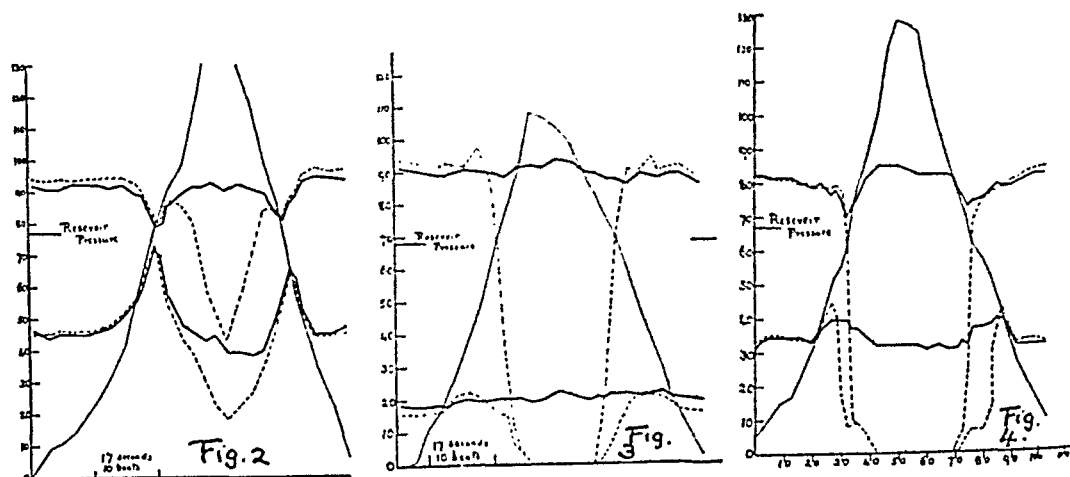


Fig. 1 Circulation Scheme



cannula inserted into a peripheral vessel in the leg; it indicated the presence or absence of a peripheral pulse but not its exact form. This method could only be used to investigate the accuracy of systolic estimates, since space did not allow the insertion of an adequate oscillometric compression system.

Standardization in the animal experiments was carried out in the usual way and corrections were made for the saline levels in the manometers and also for variations in the position of the limb from the horizontal.

c. In experiments on man the triple bag system with optical recording was used on one arm, and the pulse below was also recorded; by a T-tube connection an ordinary armlet on the other arm was inflated at the same pressure, and the various stages of auscultatory sounds were signalled.

Fig. 1. Schema: water entered from a reservoir through a mechanically operated valve *T*; light rubber tubing of 8.3 mm. bore with 0.03 mm. wall and 27.5 cm. in length was used for section *a*; heavy walled tubing of 6 mm. and 2 mm. wall for all other connections with lengths—*b*—3.5 cm., *c*—1.0 and 3.5 cm., *d*—3.5 and 3.0 cm., *e*—4.5 cm., *f*—3.0 and 4.0 cm. Tube *O* connected the compression chamber with the oscillometer, tube *R* with an air reservoir, the outer chamber of the oscillometer, the manometer and the supply of compressed air. The outflow tubes were packed with cotton, and controlled by screw clips.

Fig. 2. Effect of compression on pressures in schema when compressed segment consisted of tubing 8.3 mm. bore, 0.03 mm. wall, of length 8 cm. The thin continuous line indicates compression pressure, heavy continuous lines—systolic and diastolic pressures recorded by the manometer on the proximal side, dotted lines—systolic and diastolic pressures recorded by the manometer on the distal side of the compression. The abscissae indicate time, the ordinates—pressure in millimeters of mercury.

Fig. 3. The effect of an experiment similar to those of figures 2 and 4, except that an excised carotid artery was used for the compressed segment, and the whole system was perfused with normal saline. Symbols as for figures 2 and 4.

Fig. 4. The effect of an experiment similar to that shown in figure 2, except that the compressed segment consisted of tubing of 4 mm. bore with wall of 0.008 mm. thickness and with an outer supporting cover of thin silk. Symbols are used as in figure 2. The outflow from tubes *c* and *d* was high as compared with that from tubes *f*. Abscissae indicate time in seconds.

Fig. 5. The pressures recorded in a dog with a section of femoral artery removed and reinserted within a compression chamber. The thin continuous line indicates the compression pressure, the heavy continuous lines the directly recorded systolic, diastolic and pressures on the proximal side of the compression; the dotted lines the systolic, diastolic and pressures on the distal side of the compression. At either end of the figures are indicated the values observed in a series of pulse cycles obtained before and after the application of the compression pressure. The open and closed circles indicate the cycles measured, since all the cycles are not recorded. Part of the actual record is reproduced in figure 8. Abscissae indicate time in seconds, ordinates—pressures in millimeters of mercury. Corrections for gravity have been applied to the values obtained in the distal manometer, as explained in the text.

Fig. 6. The pressures obtained after the production of aortic regurgitation in the experiment illustrated in figure 5. The symbols used are the same as for figure 5. Part of the record is reproduced in figure 9.

In other experiments a single receiving tambour at the elbow below the triple bag was connected not only to a recording Frank capsule but also to a stethoscope, so that both sets of observations were made on the same arm. The results obtained by both methods were similar.

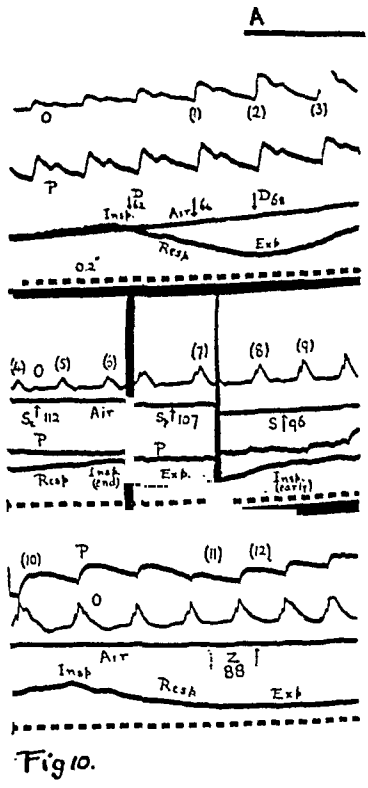
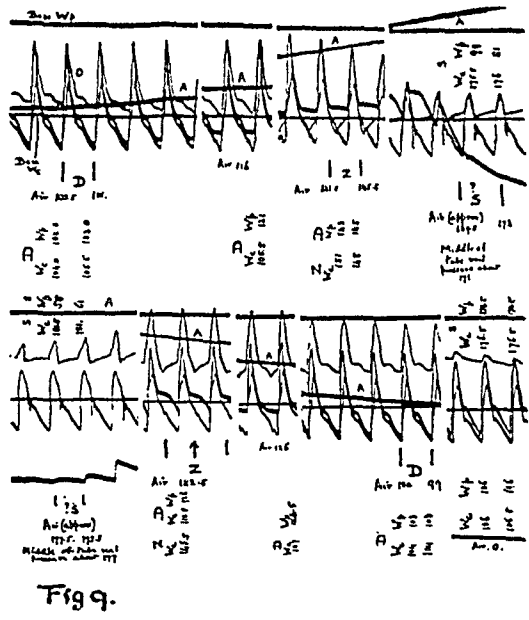
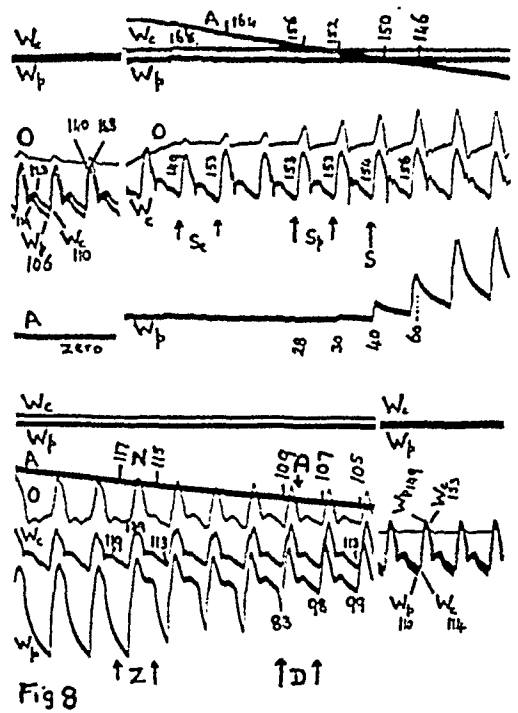
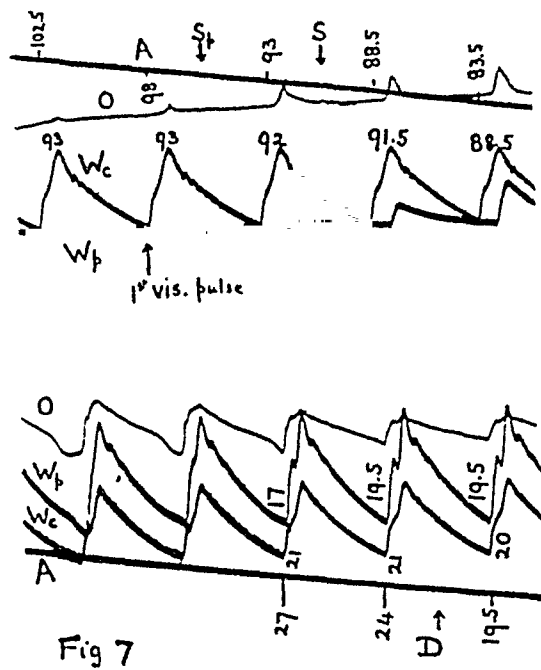
RESULTS. *Pressure changes induced by compression.* *In the schema:* The results obtained are illustrated in figures 2 to 4 and 7. Systolic pressure rose considerably above the static reservoir pressure of some 70 mm., even though the velocities of fluid movement were not high (mean velocity of systolic inflow about 20 cm. a second). During compression there was a marked fall of systolic and rise of diastolic pressure at compression pressures above the diastolic level, and the effect was the greater the larger the volume of the compressed segment. When the compression pressure exceeded the systolic level, this effect changed to one in which the systolic pressure was somewhat raised. There was no evidence of flow along the angles of the compressed segment except with the thicker tubing (fig. 2), where separation of the two sections was never complete. When the compression separated the two sections of the schema for some part of the cycle, the pressure changes proceeded quite independently in the two sections, and the rise in diastolic pressure might be more in evidence in the peripheral section. This difference between the two sections could be modified by altering the rate of outflow in the tubes; the increase was greater in the peripheral section than in the central when the rate of outflow centrally (c and d) was much more rapid than peripherally (f). A recordable peripheral pulse persisted until the compression pressure exceeded the initial lateral systolic pressure by some 3 or 4 mm., though it was obliterated at a pressure several millimeters below the maximum "end" systolic pressure. On visual examination, when the compression pressure exceeded this maximum systolic pressure, a slight bulging of the upper conical end of the compressed segment with each pulse beat could be observed; as the pressure was allowed to fall below this systolic level but when it was still above the lateral systolic level before compression, the wave was able to pass the compressed segment as a small "bolus" of stream-line shape which only partially filled the compressed segment. Such pulsations represented small volumes which were inadequate to give a recordable pulse below the compression, but they were indicated by a quite definite increase in the oscillometric pulsation. Part of an actual record obtained with a segment of a dog's carotid artery within the compression chamber, reproduced in figure 7, shows the development of an increased oscillometric change as the "bolus" type of wave was transmitted, the later appearance of a recordable peripheral pulse (both of which changes may develop at compression pressures above the true lateral systolic pressure) and the production of a peripheral pulse pressure which at moderate compression considerably exceeded the central.

In dogs: The essential changes are illustrated in figures 5 and 6, which show the pressures recorded in a dog with a glass chamber inserted in the thigh; figure 5 shows the results in the normal dog, figure 6 those obtained later after the production of a moderate aortic regurgitation by the method previously described (Bazett, 1924). Parts of the actual records are shown in figures 8 and 9. The differences between the two sets of data depended on the aortic lesion, since intermediate changes were obtained in the period between these records with a smaller aortic leak. The peripheral end of the thigh was somewhat higher than the central, and the values of the peripheral manometer have been corrected for this gravity effect; the actual values were 4 mm. lower than those charted.

Though complicated by rhythmical physiological variations the figures demonstrate the presence in the animal of factors similar to those acting in the schema. In both there was a lowering of systolic pressure during compression, a peripheral pulse was recordable even when the compression pressure exceeded the pressure recorded by the central manometer, and the "bolus" type of wave transmission continued even when the compression pressure exceeded that indicated by the central manometer by 21 mm. (figs. 5 and 8). Neither before nor after aortic regurgitation was there any definite increase in the centrally recorded systolic pressure at the highest compression pressures, but the transmission of the pulse waves was never entirely prevented. Reflected waves produced only minor variations of the systolic and diastolic waves, though at pressures just below the systolic level a marked negative reflection, creating an apparent deep incisura of peripheral origin, was a conspicuous feature of the centrally recorded pressures, and this wave appeared to contribute to the formation of a negative wave in the oscillogram.

Neither before nor after aortic regurgitation was the central diastolic pressure much affected by compression. In the normal animal the peripheral diastolic pressure fell rapidly and earlier than the systolic, so that the peripheral pulse pressure was increased; but after aortic regurgitation the peripheral diastolic pressure was raised enormously, and the peripheral pulse pressure reduced.

A marked slowing of the pulse wave transmission, such as has been described by others, was always seen, and often a sharp negative wave preceding the main upstroke of the peripheral pulse. Such waves may be clearly seen in the original of figure 9 at compression pressures above the diastolic level, and are also just detectable in figure 8 at pressures between the diastolic level and 140 mm., except that during deflation at the diastolic level these waves are masked by a negative wave of much longer duration and of quite different origin. This longer wave also often occurred in the schema and by the use of single pulses could be demonstrated to belong to the end of diastole, and not, like the other, to the beginning of systole.



Riva Rocci criteria. Diastolic pressure: The essential criteria were changes in the character of the oscillogram, and in the peripheral pulse, and the development of an initial negative wave in the latter under favourable conditions. The changes in the oscillogram may be seen in figure 7 which shows a record obtained in the schema and, therefore, uncomplicated by physiological variations. In the earlier pulses of the lower half of the figure the flattened area at the end of the cycle, dependent on the presence of a flat empty tube, is a conspicuous feature of the oscillogram; at the third pulse this disappears, and the more rapid rate of fall that precedes it only survives. Under conditions when the compressed segment was partially collapsed at the end of a cycle, the following pulse produced a much more rapid volume change at its commencement and, consequently, a much more rapid upstroke of the oscillogram; this change disappears only at the last pulse of figure 7; the air pressure of 24 mm., therefore, exceeded the true pressure and that of 19.5 was below it, as indeed was the case.

Fig. 7. Part of an actual record obtained in the schema with compression of a segment of carotid artery within the chamber. *A* indicates air pressure, *O* the oscillogram, *W_c* the Wiggers manometer central to the compression, *W_p* that peripheral to the compression; *Sp*, *S* and *D* indicate the position of indirect estimates of systolic and diastolic pressures by the criteria discussed. The values indicate the pressures in millimeters of mercury of the air pressure, and in the upper section of systolic pressure in the near manometer, in the lower section of the diastolic pressures in the two manometers. The appearance of the first visible pulse of the "bolus" type is also indicated.

Fig. 8. Part of the actual record of the experiment plotted in figure 5. *W_c* indicates the arbitrary base line and record of the centrally placed Wiggers manometer, *W_p* the base line and record of the peripheral manometer; *A* indicates the air pressure, and its zero is shown in the first section obtained before compression; *O* is the oscillogram. The numbers indicate the values of pressures at various points. The marks *Sc*, *Sp*, *S*, *Z* and *D* indicate the points at which the various criteria for systolic, dicrotic, *Z*, and diastolic pressure were considered to occur. Visible pulsations of the "bolus" type appeared to pass the compressed segment, even at pressures above 166, but they were extremely small. The apparent development of the peripheral pulse before that of the central is due to parallax.

Fig. 9. Part of the record of the experiment plotted in figure 6. The oscillogram and part of the air pressure record has been slightly retouched to assist reproduction. The figures given are inserted above or below the records with a letter to indicate the phase of the cycle measured. Remarks as to the probable pressure at the center of the compressed segment represent estimates based on corrections for gravity and an assumption of the loss of 2 mm. pressure through friction.

Fig. 10. Part of record of air pressure, peripheral pulse, oscillogram, and respiration on compression and decompression of the arm in man obtained with more rapidly moving paper than that usually employed to illustrate the shape of the waves. The air pressure and respiratory records are so marked, *O* is the oscillogram, and *P* the peripheral pulse below the cuff. The small pulse (11) is seen to depend on the lowered systolic pressure level of early expiration.

The situation is somewhat more complicated in the dog. Thus in figure 8 it may be observed that at a compression pressure of 109 mm. the oscillogram showed the increased rate of fall, after demonstrating the flat area in the two previous pulses; the peripheral pulse showed no true negative wave of short period but did show an abnormally abrupt fall of pressure at the end of diastole. This wave of diastolic origin probably depended on the relation of outflow to elasticity constants in the peripheral section, when this section was separated from the rest of the system by the closed and empty compressed segment. Both these criteria disappeared at a pressure of 107, and the diastolic pressure was estimated as 108 mm. The pressure recorded by the central manometer was 113 but since there was, in the absence of compression, a fall of pressure of 8 mm. from gravity and friction along the compressed segment, there was no evidence of any error. The presence at this time of a very low diastolic pressure in the distal segment did not interfere with the accuracy attained.

After the production of aortic regurgitation (fig. 9) a compression pressure which somewhat exceeded the diastolic pressure instead of producing in the oscillogram a flattened terminal area at the end of diastole produced, on the contrary, only an increased rate of fall, and the delay in the production of the flattened area seemed to vary with, and be dependent on, the increase observed in the peripheral diastolic pressure. At the true diastolic level there was a very definite and abrupt development of an initial negative wave of short duration immediately preceding the upstroke of the peripheral pulse, as well as a less definite increased abruptness of the oscillographic upstroke. These criteria were uninfluenced by the peripheral increase of diastolic pressure; they may be seen more clearly in records obtained on man with a somewhat increased rate of paper movement (fig. 10). The occurrence of the rapid upstroke of the oscillogram is seen in pulse 1, the initial negative wave is just detectable in the peripheral pulse in pulse 2, and in the oscillogram in pulse 3. An initial slow upstroke at the start of the oscillogram may also be noted; its origin may be in the upper cone of the compressed segment. The diastolic pressure can be estimated as between 62 and 64 mm. on the basis of the rapid oscillographic upstroke preceded by a slurred start, and as between 64 and 68 mm. on the basis of the development of a negative wave.

The schema and animal experiments do not permit any decision as to which is the more accurate criterion, for in neither type of experiment is there present within the compressed area a series of vessels of different size with presumably somewhat different internal pressures. Normally in man the two criteria occur at pressure levels which are almost identical (see table 3) but recent unpublished observations (Bazett, Scott, Maxfield and Blithe) indicate that under certain conditions they may develop at defi-

nately different pressure levels, and the increase in steepness of the oscillographic curve may be rather gradual. Mainly on theoretical grounds the diastolic pressure has, consequently, been estimated in man by first noting the lowest compression pressure at which a peripheral negative wave is developed (either in the oscillogram or peripheral pulse) and then determining the nearest pressure below this at which the oscillogram attains its maximum steepness of upstroke. Diastolic pressure is taken as the mean between this compression pressure, and that at the next pulse.

The accuracy of the estimates in a series of experiments in the schema and on animals, where these complications are absent, is indicated in tables 1 and 2. In table 1 selected data from schema experiments are given, which demonstrate that the speed of inflation or deflation (nos. 1-3) has no great effect on the accuracy of the mean of the compression and decompression estimates. In table 2 data obtained in experiments on dogs are given without distinction between compression and decompression; in this table the values included under "range" and "mean" represent the direct estimates made before and after compression of the limb; those labelled "simultaneous" indicate the range of directly measured pressures at the time the indirect estimates were made; the indirect estimates are the mean values of all estimates made during compression and decompression. The average error in the estimate of diastolic pressure was in the schema experiments ± 2 mm., and in the animal experiments ± 1 mm. The estimate was in the schema usually about 1 mm. too high, in the animal experiments 1 mm. too low.

The estimation of *dicrotic pressure* could not be made in the schema. In dogs it was indicated in the oscillograms by a dicrotic wave which arose from the descending limb of the main pulsation, and which grew in size as the air pressure was lowered (fig. 8); at pressures slightly below the dicrotic pressure, a dicrotic wave also became evident in the distal pulse. Both in animal experiments and on man there may be developed, during compression, a secondary wave which resembles a dicrotic wave; such a wave may be seen in figure 10 in the oscillograms of the first two waves of the lowest section (pulse 10). The most marked and earlier of the waves in pulse 10 is certainly not a dicrotic wave, but an effect of the compression system; the less marked later wave on the down-stroke may or may not be a dicrotic wave; its fading out as the pressure falls and absence in pulse 11 does not exclude it being a dicrotic wave, since there were marked respiratory variations in pressure in this experiment. Probably this latter wave represents a true dicrotic wave, and figure 10 illustrates the difficulties that may be encountered in determining the presence or absence of a dicrotic wave when there are marked respiratory variations in pressure. When the dicrotic notch is very marked it is probable that the compressing pressure may be below that of the dicrotic wave even when this wave

TABLE 1
Indirect measurements of lateral pressure in the schema

NO.	COMP. TUBE	COMP. OR DECOMP.	UNON-STRUCTURED PRESS	SIMULTANEOUS COMPARISONS								REMARKS
				Systolic						Dia- stolic		
				Direct	Se	Direct	Sp	Direct	S	Direct	Est.	
1	Rubber	Comp.	81/32	83	90	78	85	74	80	32	34	Rapid comp. and decomp. Mean ests: Se. 87.5. Sp. 82.5. S. 75.5. D. 31.5
		Decomp.	81/32	84	85	84	79	78	71	32	29	
2	Rubber	Comp.	82/34	85	83	76	81	73	75	34	33	Slower comp. and decomp. Mean ests: Se. 85. Sp. 80.5 S. 73. D. 31.5 (See fig. 4)
		Decomp.	82/32	85	87	80	80	76	71	35	30	
3	Rubber	Comp.	81/35	84	83	76	76	76	74	37	35	Still slower comp. and decomp. Meanests: Se. 82.5. Sp. 76. S. 72. D. 35
		Decomp.	81/35	84	82	82	76	77	70	37	32	
4	Rubber	Comp.	90/33	94	90	87	84	87	83	35	36	Slow comp. and decomp. Mean ests: Se. 90.5. Sp. 84. S. 81. D. 36
		Decomp.	90/33	94	91	87	84	87	79	38	36	
5	Rubber	Comp.	87/4	100	105	97	94	94	88			Very slow comp. and decomp. Very open outflow. Mean ests: Se. 101.5. Sp. 88.5. S. 87.5
		Decomp.	87/4	100	98	93	83	93	87	4	8	
6	Rubber	Comp.	103/16			108	101	106	103			Slow fluctuations of air pressure at systolic level. Very open outflow. Mean ests: Sp. 101. S. 103
		and de- comp.				107	105	107	105			
7	Artery	Comp.	89/18			90	85	90	85	18	19	Rather rapid compression. Mean ests: Se. 93. Sp. 87. S. 87. D. 19.5
		Decomp.		94	93	90	89	90	89	21	20	
8	Artery	Comp.	90/20			92	(100)	92	(100)	11	(16)	Rapid compression and these values doubtful. Mean ests: Se. 93. Sp. 96. S. 91. D. 22
		Decomp.		94	98	93	96	92	91	21	22	
9	Artery	Comp.	93/13			92	94	91	85	12	15	Mean ests: Se. 99. Sp. 93.5. S. 89. D. 17.5
		Decomp.		93	99	93	93	93	93	17	20	

rises from the base line of the oscillogram, and that a duplication of its peak (Bazett and Laplace, 1933b) should, in this case, be taken as the criterion. This condition has not, however, been duplicated in animal experiments.

The mean discrepancy of the estimates from the mean value of the di-crotic wave before compression was in the experiments listed in table 2 ± 4 mm., with the average estimate some 2 mm. too high.

The estimation of *end systolic pressure*, from the occurrence of a sudden increase in the size, and change in character, of the oscillometric curve,

TABLE 2

NO.	SYSTOLIC			DICROTIC		DIASTOLIC		REMARKS
	Direct Est.	Indirect Est.		Direct Est.	Indirect Est.	Direct Est.	Indirect Est.	
		Sp.	S.					
1.	Range.....	102-115			91- 97		80-86	Artery in chamber branches tied
	Mean.....	109			95		84	
	Simultaneous...	101-110	107	100	90- 97	97	80-86	
2. (a)	Range.....	136-152			115-126		103-112	As in 1
	Mean.....	145			121		108	
	Simultaneous...	149-153	156	151	115-120	119	107-109	
(b)	Range.....	149-158			120-126		107-111	As in 2(a). (See fig. 8)
	Mean.....	153			123		109	
	Simultaneous...	142-153	153	149	117-119	121	107-110	
(c)	Range.....	156-164			107-111		92- 98	As in 2(a) and 2(b) but with moderate aortic regurgitation
	Mean.....	159			109		95	
	Simultaneous...	151-161	153	149	110-116	114	96- 97	
(d)	Range.....	173-188			130-144		99-104	As in 2(a) to 2(c) but with greater degree of regurgitation (See fig. 9)
	Mean.....	180			137		102	
	Simultaneous...	174-180		174	144-149	143	102	
3. (a)	Range.....	145-176						Artery intact. Branches not tied. Big resp. variations made comparisons difficult
	Mean.....	159						
	Simultaneous...	153-182	163					
(b)	Range.....	142-196						As in 3(a) but with aortic regurgitation
	Mean.....	180						
	Simultaneous...	190	185					
4. (a)	Range.....	159-193						As in 3(a). Irregular fluctuations in blood pressure
	Mean.....	?						
	Simultaneous...	158-173	160	160				
(b)	Range.....	149-160						As in 4(a) but with aortic regurgitation
	Mean.....	155						
	Simultaneous...	152-157	154	151				

can be made accurately, as was previously reported by Bazett and Laplace (1932a); in the schema the indirect estimates averaged 1 mm. too high, and the mean variation from the direct estimate was ± 3 mm. The estimation of *lateral unobstructed systolic pressure* was difficult owing to the complexities introduced by the changes in the real pressure as the result of the compression. At compression pressures somewhat below those

giving the end systolic pressure criterion a recordable peripheral pulse was developed, a criterion designed Sp in the tables; this criterion might be developed at compression pressures either above or below the true lateral systolic pressure according to the relative values of the varying sources of error. Thus in the schema experiments (table 1) the mean discrepancy was ± 3 mm., and the mean of the estimates was 1 mm. too low, while in the dog experiments (table 2) the mean discrepancy was ± 4 mm., and the mean of the estimates was 2 mm. too high. The criterion previously suggested by us for lateral systolic pressure, namely, the presence of a shoulder on the oscillogram or on the peripherally recorded pulse gave values which were too low both in the schema and in the dog; thus in the schema (table 1) these estimates had a mean value 3.7 mm. too low, and

TABLE 3
Comparison of auscultatory and optical estimates

SUN.	SYSTOLIC				DIASTOLIC					
	Opt. Sp.	Auscult. syst.	Discrepancy		Opt. neg. wave	Opt. oscill.	Auscult. fading.	Auscult. disapp.	Discrepancy*	
			Mean	Extreme					Mean	Extreme
1	115.0	115.0	0	0	58.5	57.3	55.0	35.5	± 4.0	+3 -17
2	118.0	116.0	-2	0 -8	71.0	67.8	67.5	47.8	± 7.0	+11 -21
3	113.0	113.5	+0.5	+2 0	61.0	58.2	55.8	40.5	± 6.2	+8 -14
4	126.7	126.2	-0.5	0 -4	78.7	77.0	82.0	73.7	± 8.3	+18 -10
5	139.7	136.4	-3.3	0 -9	69.3	67.3	59.3	40.5	± 10.7	+9 -23

* Discrepancy estimated between optical oscillometric and auscultatory fading.

nearly all the discrepancies were negative, while in the dog experiments the mean value was 5.3 mm. too low and again nearly all the discrepancies were negative.

Comparison with auscultation criteria. The auscultatory criterion for systolic pressure checked well with that of the first recordable peripheral pulse (Sp). There was rarely a discrepancy of more than a single cycle, which, with the slow pressure changes used, only involved differences of 2 or 3 mm. The "lateral systolic" criterion (S) occurred at pressure levels where the sounds became much louder or assumed a murmur character. With slow compression or decompression the sounds changed gradually and showed respiratory variations, and the observer had considerable difficulty in analysing the changes, so that exact comparison was difficult.

Table 3 gives values for systolic pressure obtained on 5 subjects by auscultation and by the appearance of a recordable pulse below the cuff (Sp criterion). The method used was that where observations were made on the same arm. The practical identity of the two estimates is obvious, and the optical record is shown to be slightly the more reliable. All the subjects were normal, except subject 5, who had aortic regurgitation.

Comparisons of the diastolic criteria were also difficult with slow pressure changes. Sounds continued, especially during decompression, at compression pressures below the diastolic pressure indicated either by the peripheral negative wave or the character of the oscillometric curve. These sounds were sometimes marked, particularly in aortic regurgitation, and caused confusion in judging the auscultatory criterion. The estimation of diastolic pressure from the fading of sounds was in fair agreement with estimates from the oscillometric curve (see table 3); in fact, if subject 5 with aortic regurgitation be excluded, the mean estimates by the two methods were almost identical. However, the optical method appeared to be the more reliable. Though some of the variations in any single subject were undoubtedly due to physiological variations, of respiratory or other origin, yet some were certainly due to experimental error; the mean deviation from the means for the different individuals, excluding the subject with aortic regurgitation, was ± 3.9 mm. for the negative wave criterion which was usually very sharp, ± 4.8 mm. for the oscillometric change which was less definite, and was ± 6.2 mm. for the auscultatory criterion. The two methods did not agree in the subject with aortic regurgitation, where the auscultatory estimate was definitely much lower. The mean deviations from the mean were in this subject (12 observations) ± 3.4 for the negative wave criterion, ± 4.6 for the oscillometric change, and ± 7.7 for the auscultatory criterion. The consistency of the optical methods was the same as, or somewhat better than, that observed in normal subjects, that of the auscultatory method was definitely less. The two optical criteria agreed well with one another in this series; the change in the oscillogram gave almost invariably values some 0 to 4 mm. lower than those indicated by the negative wave.

DISCUSSION. The complexities introduced into a pulsating elastic system filled with fluid when a segment is compressed appear to depend on several factors; of these one, long recognised, namely, the development of either positive or negative reflected waves appears to play a minor rôle except at the systolic level; a second, which, though obvious, seems to have attracted little attention, is the fact that when the segment is closed the two parts of the elastic system are completely separated, and the pressure changes in each part develop separately according to their own conditions such as rate of outflow and volume elasticity coefficient; a third factor is a decrease in the volume elasticity coefficient of the system as a

whole, as the distensibility of the compressed segment becomes determined by the air column rather than by the vessel wall; this factor has long been recognised in relation to its effect on the pulse wave velocity, but its effect on the pressures developed has received little attention; a fourth factor is the alteration of the wave form by the development of "breakers" as the result of the great contrasts in the speed of transmission of different parts of the pulse wave.

Reflected waves. While a negative reflection of the pulse wave is a conspicuous feature at compression pressures just below the systolic level when the peripheral vessels are empty, positive reflections of the same wave do not develop rapidly at higher compression pressures nor are they large. The air system which leaves an open cone at the upper end of the compressed segment appears to act as a cushion which prevents any sudden reflection of the wave, particularly when the compression pressure does not much exceed the systolic level. Consequently, the differences between end and lateral pressures recorded by the central manometer were not as great as those observed when the artery was closed by a clip (Bazett and Laplace, 1933a). Though the minimizing of this effect may have been partly due to the cannula holding open the upper cone, and to a diminution of the velocity head by the ligation of branches, the differences between end and lateral systolic pressures were still subnormal, even when these factors were excluded by the use of a somewhat different system (table 2), so that at least part of the minimising effect may be considered as a concomitant of all Riva Rocci systems.

The independence of pressure changes in the separated sections and general effects on distensibility. These two factors are closely related and may be considered together. In systole the system reacts as a whole, and if the compressed segment is open sufficiently long the systolic pressures of both sections are the same; the effect of the air column on the volume elasticity coefficient, however, damps the systolic wave and lowers systolic pressure. In the diastolic phase the two sections are separated by the compressed segment, and the pressure changes develop independently in the two sections according to their own rates of outflow and volume elasticity coefficients. If the compressed segment is closed early in diastole, the duration of diastole is thereby prolonged in the distal segment, and diastolic pressure consequently tends to fall to a lower level, so that the peripheral pulse pressure is usually greater than the central. Since the volume elasticity of the compressed segment is the lowest of the whole system, at one stage in the cycle it tends to empty its contents rapidly and to supply for a while the loss of the system as a whole, while maintaining a temporary plateau in the pressure curves. According to the conditions in the system this discharge of the contents may occur mainly into the central vessels or mainly into the peripheral, and this factor is concerned in the effect of vary-

ing the rates of outflow centrally and peripherally on the relative changes in central and peripheral diastolic pressure in the schema. The effect produced on diastolic pressure varies with the volume of the compressed segment, but a volume, which may be quite ineffective if discharged into a large central system, may produce considerable changes, if directed into the smaller peripheral section. The data that have been given are all explicable on this hypothesis.

In the experiment of figure 5, the slight upward slope of the limb would tend to cause closure of the compressed segment first at its peripheral end, and the consequent central discharge of the contents of the single artery would not alter pressures in the large central reservoir appreciably; on the other hand, after production of aortic regurgitation the rapid central fall of pressure would tend to cause closure at the central end and discharge of the contents in the opposite direction peripherally, where the contained volume would be by no means negligible. The marked increase in the peripheral diastolic pressure of figures 6 and 9 would depend then on two factors: *a*, this peripheral section was filled in systole at the high systolic pressure which resulted from aortic regurgitation, but during diastole it was completely separated by the compressed segment from any diastolic effect of aortic regurgitation; and *b*, this section received the contents of the compressed segment as was indicated by the maintenance of the peripheral diastolic pressure at a plateau which had the same pressure (after subtracting the 4 mm. gravity correction) as the air system, while the oscillometric curve demonstrated that the compressed section at this time was discharging and had not emptied. In contrast, figure 8 shows evidence of only a very small and brief peripheral discharge of the contents. This effect of the discharge of the contents may be recognised in most of the graphs in that it tends to raise the diastolic pressure parallel with the change in the compressing pressure, as must be the case since the latter supplies the expelling force.

These factors must be quite important even in the indirect estimation of pressures in an intact animal, for the pressure changes reported previously in the opposite femoral of a dog during the compression of a thigh (Bazett and Laplace, 1933a; fig. 3) are also most readily explained on the same hypothesis. The fall in systolic and rise in diastolic pressure observed during compression in the normal condition should be expected, since the volume contained in the compressed thigh would be considerable, and in the normal sloping position of the thigh the contents would be discharged centrally, and should be effective in altering central diastolic pressure; on the other hand, after aortic regurgitation the systolic effect should be preserved, but the contents of the compressed thigh should be directed peripherally and be quite ineffective in altering central diastolic pressure. The data of both experiments, however, imply that in the thigh the pres-

sure fall at the central end of the femoral artery with aortic regurgitation is much more rapid than at the peripheral end of the same artery, and that there is a considerable retrograde flow in diastole, even though in the experiment here reported there was only a hole about 6 mm. in diameter in a single aortic cusp.

Alterations in wave form. The formation of secondary waves, which precede the main pulse and which typically include a sharp negative deflection, has been ascribed by Bramwell (1925) to the formation of breakers as the result of the very different rates of transmission of different parts of the wave. This hypothesis is supported by the observations of Frank and Wezler (1931) that the rate of transmission of the main wave is, at compression pressures above the diastolic level, less than 1 meter a second. According to Bramwell's hypothesis, these secondary waves should develop more readily the steeper the upstroke of the pulse and the greater the differences between the transmission rates of the different parts of the pulse curve. In favour of Bramwell's hypothesis are the following observations: no negative wave of this type was developed in the schema where the initial development of pressure occurred at some 300 to 350 mm. Hg per second; negative waves were developed in the normal dogs where the initial pressures developed at some 800 to 900 mm. per second but these waves were slight, and usually disappeared again at compression pressures well above the diastolic level; the negative waves were well developed and persisted over a wide range of compression pressure in dogs with aortic regurgitation in which the pressure rise developed at 1100 to 1800 mm. per second; in man the development of these negative waves has been more marked in older subjects, where greater contrasts in pulse wave velocities of the compressed and uncompressed vessel are to be anticipated; the common disappearance of a negative wave at the higher compression pressures might depend on the vessel wall attaining, under these conditions, only a low tension and a relatively low rate of wave transmission.

It was suggested by Bazett and Laplace (1933b) that pulsations could not pass a cuff unless the pressure remained above the compressing pressure long enough to allow the wave to complete the passage of the compressed segment. Erlanger (1916) considered that passage of fluid along the angles of a compressed tube could occur even in arteries. Both these assumptions were found to be untrue.

Accuracy of indirect estimates of lateral pressure. At the lateral systolic level the compression system introduces two errors of opposite sign, one a rise in systolic pressure from a positive reflection of the wave, the other a fall due to an increased distensibility of the system; at this level both of these errors are minimal, and tend to neutralise one another, so that the actual pressure is not much altered. The oscillometric excursion is increased, by the "bolus" type of wave described, at considerably higher

pressures which correspond with end pressures, the lateral systolic criterion appears to be developed at pressures about 5 mm. below the true lateral systolic pressure. A recordable peripheral pulse is developed at an intermediate level, and in these experiments at one which only slightly exceeded the true lateral pressure, but the error of such a criterion is apt to vary with the sensitivity of the recording system, and its ability to demonstrate the presence of the bolus type of wave. The tambour system used on man is slightly more sensitive in this regard than auscultation, while auscultation appears to be more sensitive than a direct recording system. For von Bonsdorff (1932), reviewing the data obtained by Wolff and himself by direct arterial puncture in man, shows that the auscultatory estimates had errors ranging from +13 to -1 mm., with a mean value 5 mm. too high, while estimates from the recurrence of a directly recordable pulse had errors ranging from +15 to -12, with a mean value 1 mm. too high. Part of the considerable discrepancies of von Bonsdorff's data may depend on the fact that simultaneous measurements, when made, involved opposite arms, and that for the most part the comparisons were not simultaneous. Combining von Bondorff's data on man with that here reported on dogs, it would appear that the reappearance of a peripheral pulse, recordable on a tambour system, is likely to have a variable error of some +5 mm. In a large number of estimations on man the differences between this "Sp" estimate and the lateral systolic estimate "S" has averaged 5 mm.; if the Sp criterion is too high, then the S criterion probably has less of a negative error than in the animal experiments, and on a priori reasoning its error is apt to be the less variable. It appears the preferable criterion.

In regard to criteria for diastolic pressure the data presented indicate that the flattening of the end of an oscillometric curve is a poor indicator either of lateral or end diastolic pressure, since its occurrence is modified by the changes in diastolic pressure, which may be produced by compression. On the other hand, the presence of an initial negative wave in the peripheral pulse appears to be quite independent of such variations in the peripheral section, but while its presence is conclusive evidence that diastolic pressure has been exceeded, its absence is less conclusive, since the wave is not always readily developed. Similarly a sharp oscillometric upstroke of maximum steepness is probably an accurate index, but an abrupt development of this change is not always obtained. The ordinary auscultatory criterion (commencement of fading), if deflation is slow, appears to be accurate *on the average* in normal subjects under normal conditions in these experiments, as in those reported by von Bonsdorff, but to be unreliable in other conditions. The criticism by von Bonsdorff of oscillatory estimates based on the size of pulsations is supported; his criticism of estimates based on the shape of waves appears unjustified for the

method he employed appears to have been inadequate to record the initial negative waves.

The work here reported was initiated as the result of attempts to correlate blood pressure changes with stroke volume (Bazett et al., 1934); it is hoped to give a fuller account of these experiments soon. It may be pointed out here that the apparent unwarranted emphasis here given to discrepancies of only a few millimeters between direct and indirect estimates is not artificial. The difference between diastolic and diastolic pressures in man is often only 15 to 20 mm., so that an error of only 2 mm. in estimating this difference may cause a 10 per cent error in such calculations.

SUMMARY

1. It has been shown in a schema, and also in dogs, that the process of compression of a segment of the vascular system by an air column alters the actual pressures within the system at compression pressures which exceed diastolic pressure. Diastolic pressure is raised particularly at pressures just above the diastolic level; systolic pressure is lowered at pressures below the systolic level, raised at pressures above it. At the true lateral systolic level these two effects more or less neutralize one another. The changes are shown to be modified by the volume of the compressed segment. The raising of diastolic pressure and lowering of systolic result from the decreased volume elasticity coefficient created by the air column; the ultimate rise in systolic pressure depends on the stoppage of the flow and reflection of the primary wave; this second change is only brought gradually into action by an air compressing system, owing to the cushioning effect of the air column.

2. The effects of compression on diastolic pressure may be very unequal in the two separated parts of the main system. When separation is effected, the pressure changes proceed independently in the two sections according to their own volume elasticity coefficients and rates of outflow. The peripheral pulse pressure tends to be the greater owing to the lengthening of the diastolic period in this section, and the consequent fall of diastolic pressure to a lower level, but the whole picture may be altered by the direction in which the contents of the compressed segment are mainly discharged. In general this discharge is towards the side which has the slower rate of fall of pressure.

3. The changes observed on compression in dogs with aortic regurgitation are shown to indicate retrograde flow in the femoral artery during diastole.

4. An oscillometric increase in pulsation is shown to be an accurate indicator of end systolic pressure; a recordable peripheral pulse occurs at pressures above the true lateral systolic level, but owing to the balance of

opposing factors the total error is usually small. The lateral systolic pressure criterion previously described occurs in dogs at pressures somewhat below the true pressure; some reasons are given which suggest that the error involved is even less in man.

5. Dicrotic pressure may be fairly accurately estimated by oscillometric methods under favourable conditions.

6. Diastolic pressure may be estimated by the development of a very rapid upstroke on the oscillogram as the vessel wall assumes a floating position; at the same or at a slightly higher pressure an initial negative wave usually precedes the upstroke of the peripheral pulse, oscillogram or both. These criteria are not affected by changes in the diastolic pressure of the peripheral section. The flattening of the final area of an oscillogram is not a good indicator of either the end or lateral diastolic pressure, as it is affected by changes in the peripheral diastolic pressure produced by compression.

7. The flow of fluid along the angles of compressed arteries is denied; fluid transmitted at compression pressures, which exceed the lateral systolic pressure, does so in the form of a wave of "bolus" type.

We would like to take this opportunity to thank Miss M. E. Maxfield and Mr. N. P. Wander for their assistance in many of the experiments and also our technical assistant Mr. Albert Afford for the skill and care exhibited in making the thin rubber tubing used in some of the experiments.

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THE ERYTHROCYTE AND HEMOGLOBIN INCREASE IN HUMAN BLOOD DURING AND AFTER EXERCISE¹

EDWARD C. SCHNEIDER AND C. B. CRAMPTON

From the Department of Biology, Wesleyan University, Middletown, Connecticut

Received for publication March 5, 1935

Regardless of the final outcome of the discussion concerning the explanation of the method of the increase in the number of erythrocytes and the percentage of hemoglobin in a unit volume of blood that occurs during physical exertion, the circumstance under which the increase is brought about and the time taken for its onset and disappearance continue to be of interest to biologists.

We present, therefore, data obtained during and after work on a bicycle ergometer and after the more general exercise of running. Attention has been centered on the variations in the content of red blood corpuscles and hemoglobin in the capillary blood of the fingers. We have used the capillary blood, in spite of the fact that gravity plays a part in the distribution of blood corpuscles. The influence of gravity is one of stagnation, which best reveals itself when the individual stands at attention. Under this condition the capillary blood from the toes is richer in corpuscles than that of the fingers or of the lobe of the ear. The blood of the lobe of the ear contains less than that of the fingers.

Arnold and Krzywanek maintain that the blood should be taken from a vein when rapid blood changes are investigated. Our own experience with blood drawn from a vein and contrasted with that from the skin capillaries is that the two methods, as far as the changes due to physical exertion are concerned, reveal the same facts. The blood drawn from the basilic vein of the arm is on the whole slightly less rich in corpuscles than is that of the capillary blood of the finger of the same arm, but the difference is by no means constant. We have occasionally found the increase in erythrocytes and hemoglobin in men after running fully as great in the venous as in the capillary blood.

The influence of the load of work. The increase in erythrocytes during exertion is regarded as a compensatory adjustment whereby the tissues are more adequately supplied with oxygen. It is assumed by Beyne, Binet and Strumza that, up to a certain limit, corpuscles are supplied by the spleen in response to the demands set up by oxygen want. Since there

¹ The expense of this investigation has been met by a grant from the Charles Himrod Denison Fund.

is a rectilinear relationship maintained between the consumption of oxygen and such factors as the minute volume of breathing, the blood flow, the pulse rate, and other conditions, it seemed desirable to determine if a similar relationship is maintained between the load of work (oxygen requirement) and the increase in erythrocytes in a unit volume of blood. That such a relationship does not exist is shown by the data in table 1. The subject, J. D., worked at well spaced intervals on the bicycle ergometer for five minutes under loads of 4000, 6000, 8000, and 10,000 foot-pounds. With a load of 4000 foot-pounds, which called for approximately 1480 cc. of oxygen per minute, no change in the content of erythrocytes and hemo-

TABLE 1
Work on bicycle ergometer for 5 minutes by J. D.

LOAD	RED BLOOD CELLS			HEMOGLOBIN		
	Rest	Exercise	Increase	Rest	Exercise	Increase
<i>foot-pounds</i>	<i>millions</i>	<i>millions</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
4,000	5.17	5.15	0	116	117	0
6,000	5.16	5.43	5.2	115	120	4.3
8,000	4.98	5.50	10.4	113	124	9.7
10,000	5.16	5.80	12.4	114	127	11.4

TABLE 2
Load of 4000 foot-pounds on bicycle ergometer, C. B. C.

TIME	RED BLOOD CELLS			HEMOGLOBIN		
	Rest	Exercise	Increase	Rest	Exercise	Increase
<i>minutes</i>	<i>millions</i>	<i>millions</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
5	5.59	5.62	0	116	116	0
10	5.95	5.80	0	119	118	0
15	6.04	6.10	1	122	124	1.6
20	5.88	6.18	5.1	118	123	4.2

globin could be demonstrated. We had one subject who showed no increase in either erythrocytes or hemoglobin even when carrying a 6000 foot-pound load for 5 minutes. After a load of work is reached that does bring out a response, then each successive increase in load further augments the erythrocyte count (see table 1); but with none of our subjects were we able to show a rectilinear or a curvilinear relationship between the increase of corpuscles and the load of work. The reason for this failure to establish a definite relationship may be found in our next observations.

Time a factor in bringing about the increase. Table 2 shows that a moderate load of work which fails to cause concentration in 5 minutes may do so if continued for a longer period. The load of 4000 foot-pounds was an easy normal-load for this subject (C. B. C.); in fact, any healthy young man

quickly enters and maintains a steady-state in oxygen intake during a long period of work with this load. From the data in table 2, it would seem that some other factor than oxygen want may provoke the increase. It will be observed that after 15 minutes the 4000 foot-pound load had slightly increased both the erythrocyte count and the hemoglobin determination, and that during the next 5 minutes the increase rose to 5 per cent.

Even with an extremely heavy load, such as 10,000 foot-pounds, the erythrocyte increase is somewhat slow in developing. In table 3 is given a typical case, in which at the end of one minute no change had occurred, while at the end of 2 minutes a 7 per cent and at 3 minutes a 13 per cent increase was observed. The 13 per cent increase in erythrocytes in 3 minutes was as large as any obtained with this subject for this load in 5 minutes of work.

TABLE 3
Load of 10,000 foot-pounds on bicycle ergometer, C. B. C.

TIME	RED BLOOD CELLS			HEMOGLOBIN		
	Rest	Exercise	Increase	Rest	Exercise	Increase
minutes	millions	millions	per cent	per cent	per cent	per cent
1	6.09	6.06	0	121	120	0
2	5.99	6.43	7.3	121	128	5.8
3	6.13	6.94	13.2	121	130	7.4

Exertions that call upon more muscles than does pedalling a bicycle ergometer may more rapidly induce an augmentation in the number of erythrocytes. Thus in table 4 are given observations on a man, (H. L.), who twice ran 100 yards in somewhat less than 11 seconds. Blood drawn immediately after the run showed an increase in the count of 7.1 per cent after the first and 9.4 per cent after the second. The runner held his breath throughout the run.

Another series of runs of 100 yards made by M. R. W. (table 4) indicates that the more strenuous the effort the sooner and the greater will be the rise in the erythrocyte count. When M. R. W. ran the distance in 16 seconds no change was found immediately after the run, but when he lowered his time to 14 seconds an erythrocyte increase of 1.8 per cent was obtained, and after he ran the 100 yards in a little less than 13 seconds the augmentation rose to 6.9 per cent.

By means of violent "running in place" in the laboratory it was demonstrated that the augmentation in the number of erythrocytes may develop some time after the exertion is over. There were 8 men who ran 14 times for 30 seconds. For these the first sample of blood, drawn within 15 to 30 seconds, gave variable results, 4 showing an increase and 8 a decrease in

the count. Of the 4 who gave the increase we may conclude that it was real, since the second samples showed a further augmentation. Of the 8 who had a decrease 4 were decidedly beyond our probable errors, showing a drop of from 5.5 to 9.6 per cent. This drop appeared to be associated with a cutaneous vasoconstriction. When this vasomotor condition passed, as it always did by the time for the second sample, the increase of erythrocytes became clear. The time of maximum increase was usually delayed beyond one minute; in 2 instances it occurred at end of the third minute, while in 6 men it occurred between the second and third minute. The data of two typical runs are given in table 5.

TABLE 4
100-yard run

TIME	RED BLOOD CELLS			HEMOGLOBIN		
	Rest	Exercise	Increase	Rest	Exercise	Increase
M. R. W.						
<i>seconds</i>	<i>millions</i>	<i>millions</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
16	5.60	5.59	0	122	123	0
14	5.60	5.70	1.8	122	125	2.5
13	5.40	5.77	6.9	115	122	6.1
H. L., held breath						
11	5.48	5.83	7.1	126	135	7.1
10.7	5.43	5.94	9.4	124	134	8.1

TABLE 5
After 30 seconds of "running in place"

SUBJECT	RED BLOOD CELLS IN MILLIONS										
	Norm.	30"	60"	1' 45"	2' 15"	3' 15"	4' 15"	5' 15"	6' 15"	7' 15"	8' 15"
N. M.....	5.40	5.37	5.86	6.24	6.34	5.60	5.44	5.53	5.18	5.24	5.21
R. B.....	5.09	5.43	6.19	6.32	5.38	5.45	5.46	5.31		5.17	5.26

"Running in place" violently for 15 seconds also gave results that corresponded with those of the 30-second runs. Usually the increase in erythrocytes develops in the post-exercise period; but in exceptional cases it is well developed in the first blood sample, drawn within 15 seconds after the exertion. In one instance the maximum concentration was obtained in the first sample. The maximum rise in the erythrocyte count after the run of 15 seconds was less than after the 30-second run and ranged from 6 to 8 per cent. In every case this was reached before or by the end of the second minute.

After the 30-second run the maximum erythrocyte increase for all cases ranged between 6 and 19.5 per cent, with 10 out of 14 cases above 11 per cent. After all of the 30-second runs the men were badly "winded." On the average the augmentation in the erythrocyte count after the 30-second run only slightly exceeded that obtained with the heaviest load on the bicycle ergometer during a period of 5 minutes of work. The highest for this load was an increase of 19.3 per cent, while the maximum for the majority ranged between 10.5 and 12 per cent.

Time of recovery. The duration of the period of high concentration of the number of erythrocytes was short after the 15-second runs, usually not over 3 minutes. After the 30-second runs the majority of cases required in the neighborhood of 10 minutes to return to the pre-exercise level. In one man, in whom the maximum occurred one minute after this exercise, the return to normal was made in 3 minutes, but all of the others required 5 minutes or more. After the heaviest load, 10,000 foot-pounds, on the bicycle ergometer the normal count was not restored in less than 6 minutes and usually required 8 or more minutes. We did not ordinarily continue sampling beyond 10 minutes.

SUMMARY

During 5-minute periods of work on a bicycle ergometer the number of erythrocytes and the percentage of hemoglobin in a unit volume of blood were not increased with light loads. With heavier loads of work an increase invariably occurred and augmented as the load of work was stepped-up.

No mathematical relationship between the increase in erythrocytes and hemoglobin and the load of work was established.

A very moderate load of work, if carried long enough, may eventually, within 15 or 20 minutes, induce an increase in the number of erythrocytes. This suggests that some other factor than anoxemia may operate to induce the concentration.

Exertions that call generally on the muscles of the body, such as in running, more promptly induce the augmentation in erythrocytes. The increase was observed immediately after a 100 yard run in 11 seconds.

Ordinarily after violent "runs in place" of 15 and 30 seconds the maximum degree of concentration is slowly reached, usually within from 2 to 3 minutes.

The time required for return to normal after running is determined in part by the duration of the effort. This was accomplished within 3 minutes after 15-second runs and required in the neighborhood of 10 minutes after the 30-second runs.

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DISTRIBUTION OF VAGUS CONTROL OF THE TURTLE HEART

HENRY M. LEE

*From the Division of Physiology, University of California Medical School,
Berkeley, California*

Received for publication February 5, 1935

Differences in the action of the right and left vagi upon the turtle heart have been frequently described (e.g., Gaskell, 1883-84; Garrey, 1911). It is generally concluded that different fibers of the vagus exert different effects upon rhythm, contraction, conduction, and excitation; these effects have been described as chronotropic, inotropic, dromotropic, and bathmotropic, respectively, by Englemann (1900). Two possibilities offer themselves: 1, there may be four different types of fibers mediating four different mechanisms; 2, a single mechanism may be mediated by fibers which are distributed to different regions of the heart. This question is of particular interest with regard to the humoral hypothesis of vagus action (Loewi, 1921), since, according to this concept, a single vagus substance would be expected to produce various effects. The following studies were undertaken with this particular question in mind.

EXPERIMENTAL. In our first experiments we attempted to study the distributions of inotropic and chronotropic effects between the two vagi. The turtles used were of two species, namely, *Pseudemys elegans* and *Clemmys marmorata*. The contractions of both auricles were recorded simultaneously upon a kymograph by means of light heart levers of equal magnification, these being attached to the apices of the auricles by light threads. The vagi were dissected out in the neck, ligated, and stimulated through platinum electrodes by an interrupted current from a Harvard inductorium.

In the species *Pseudemys elegans* the right vagus caused cardiac standstill with moderate strength of stimulation in three out of four experiments; in the fourth experiment standstill resulted with strong stimulation. The left vagus produced only a slight slowing in three cases with all strengths of stimulation. The results with the second species, *Clemmys marmorata*, were similar to those mentioned above, but in five cases out of six strong stimulation of the left vagus caused complete cessation of the beat in a manner similar to the right vagus. Thus there appears to be a considerable difference between species in the distribution of fibers mediating chronotropic effects.

Since the stimulation of the right vagus usually stops the rhythm of the entire heart, any effects upon the amplitude of contraction which might take place during the stimulation are masked by the destruction of this rhythm. Accordingly, it was necessary to provide some means by which contractions could be maintained during right vagus stimulation. To accomplish such a result a Gaskell heart clamp with jaws protected by means of adhesive tape was placed at the sino-auricular junction and pressure gradually applied at this point. When the pressure was sufficiently great, the auricles ceased to respond to the impulses arriving from the pacemaker, and rhythmically occurring single induction shocks were then applied to the ventricle at rates approximating the normal rhythm. These were obtained from a second inductorium, whose primary circuit was made and broken at regular intervals by a rotary contact maker. These shocks caused regular contractions of the ventricle, followed by contractions of both auricles. Thus a ventricular-auricular rhythm was maintained. It has been demonstrated by Garrey (1911) that compression at the sino-auricular junction in the turtle does not destroy the vagus fibers, although preventing conduction through the tissue itself; and it was thus possible to observe any effect upon the contraction of the auricles during vagal stimulation. Previous to the clamping off of the sino-auricular junction, observations were made as to any changes on the rate of the normally beating heart which might be produced by either vagus.

In both species, under the above conditions, stimulation of the right vagus caused a diminished amplitude of contraction or negative inotropic effect in both auricles; figure 1 illustrates this clearly. Figure 2, which is the record of the same heart beating at its own rhythm, demonstrates the fact that ordinarily during right vagus stimulation such an inotropic effect is masked by the cessation of the rhythm. This is especially marked in the left auricle where the first contractions are actually greater in amplitude upon resumption of the beat, probably due to the greater recovery period during quiescence (see Blum, 1927).

Distribution of the vagi to the two sides of the heart appears somewhat variable. In four individuals of species *Pseudemys elegans*, the stimulation of either the right or left vagus caused a diminution of contraction which was greater in the homolateral auricle. This occurred even in the case of fairly weak stimuli. In species *Clemmys marmorata* the results were more varied. In three of four animals the right vagus produced a greater diminution of contraction in the homolateral auricle, but in the fourth case the contralateral diminution was greater than the homolateral. The stimulation of the left vagus in two of the four experiments, produced a greater contralateral diminution of contraction, but the remaining cases showed the homolateral effect of the left vagus to be the greater.

Although the total number of our experiments is not great, the results

on the artificially driven hearts show that, generally, the negative inotropic effect of each vagus is greatest on the auricle on the same side as the nerve stimulated, as observed by Garrey (1911) and Cruickshank (1920, 1921). However, it must be noted that in eight experiments there were three exceptions to this generalization, and we may therefore assume that variations are common. A comparison of the inotropic effects of both nerves indicates that the left nerve appears to exert on its own side a greater negative inotropic effect than that exerted by the right vagus on either side.

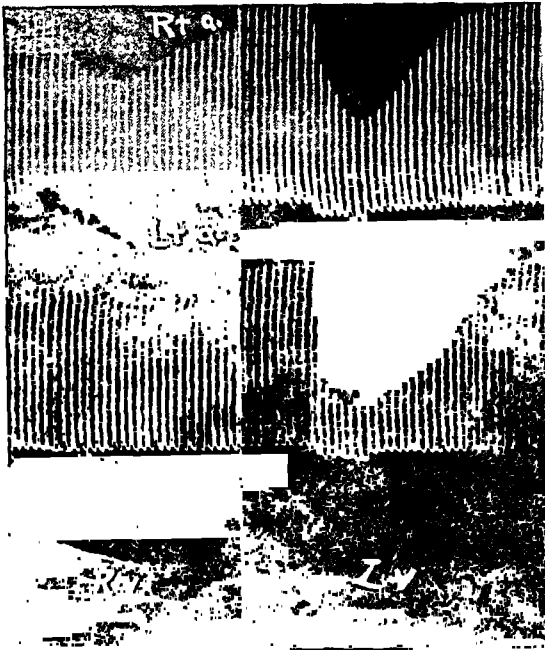


Fig. 1

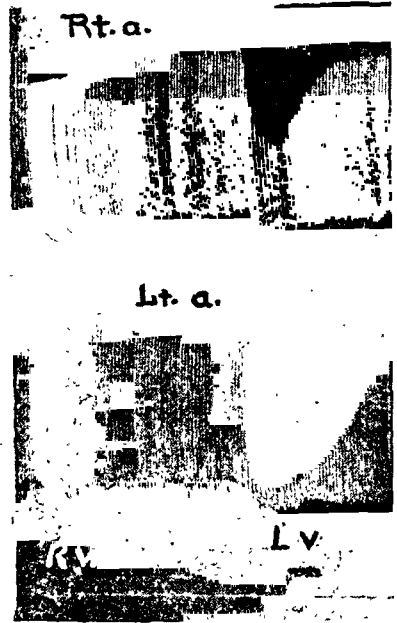


Fig. 2

Fig. 1. Contractions of artificially driven turtle auricles (*Clemmys marmorata*). Rt. a., right auricle; Lt. a., left auricle; R. v., stimulation of right vagus; L. v., stimulation of left vagus.

Fig. 2. Contraction of turtle auricles following normal rhythm from the sinus. Rt. a., right auricle; Lt. a., left auricle; R. v., stimulation of right vagus; L. v., stimulation of left vagus.

An example of this may be seen in figure 1. This must be cautiously considered, however, since it is possible that the pressure of clamping the sino-auricular junction might have caused a partial injury to the vagus fibers, particularly those of the right vagus. However, it can be seen from an inspection of figures 1 and 2 that in the case of the left vagus there was no apparent injury to the vagal fibers during the application of the clamp as judged by the magnitude of the inotropic effect of the left vagus before and during artificial rhythm.

In a second group of experiments, the threshold of stimulation of the chronotropic and inotropic effects in the two vagi were studied after the method of Gilson (1930). The species employed in these studies was *Clemmys marmorata*. A neon tube oscillator, whose output and rate of oscillation could be controlled, was employed for stimulating the vagi; the particular circuit has been described by Lange (1931, 1932). The stimulating rates most frequently used varied between six and fourteen shocks per second.

In these experiments the difference in the actions of the right and left vagi on the beat of the sinus and upon conduction over the sino-auricular junction was first studied. Each vagus was stimulated separately with currents first below the threshold of any effect and gradually increased to maximum output of the machine; the changes in rate and sino-auricular interval which were apparent by direct observation of the beating heart were noted in the order in which they appeared. A few attempts to make kymographic records of the contractions of the sinus showed that the act of attaching threads to the sinus in some way interfered with the inhibition of the pacemaker mechanism, since direct observation of changes in the contraction rate differed from the changes recorded when the sinus was attached to a lever. Furthermore, the vagal chronotropic effects were not so pronounced, nor were sino-auricular interval changes so evident.

Twenty-one different animals were examined in this group. In approximately half of the vagus nerves the chronotropic and dromotropic effects had identical thresholds; i.e., as the current was gradually increased, the decrease in the rate of sinus beat, and increase in sino-auricular interval occurred simultaneously. In the other half of the nerves the chronotropic effect had a lower threshold except in two instances, both left vagi, in which the dromotropic effect had the lower threshold. In two of these it was possible to produce slowing of the sinus beat with the maximum current output of the apparatus.

The lengthening of the sino-auricular interval was usually followed by sino-auricular block, thus producing a greater *apparent* chronotropic effect in the auricles and ventricles than in the sinus.

In a majority of cases the sinus could be brought to complete standstill; this was more common with the right than with the left vagus. An interesting phenomenon discovered was the fact that, although the pacemaker could be completely inhibited by either vagus so that the whole heart was quiescent, a mechanical stimulus applied to the sinus would cause that organ to contract, and if inhibition had not interfered with conduction this contraction would be followed by the rest of the heart in sequence. This indicates that conduction over the whole heart is still possible in spite of the vagus action.

Additional experiments were performed to test the action of stimulating

currents of various strengths on the inotropic and chronotropic effects produced in the auricles. In the first few experiments the beat of only one auricle was recorded. However, since the inotropic action of either vagus is greatest on the homolateral side, the homolateral auricle should be the better index of any changes in contractility during the stimulation of the vagi, and for this reason in the latter experiments the contractions of both auricles were recorded. The procedure followed was similar to that used above. While one or both auricles were recording on a kymograph by means of light isotonic heart levers, the vagus nerves were stimulated separately with sub-threshold currents. Next the strength was increased until either inotropic or chronotropic action occurred. Then the stimulus was increased until no further depression of contractility or rate was noted in the auricles.

Eight different animals were utilized in these experiments. In all but two nerves the inotropic effect had the lower threshold on either the homolateral or contralateral auricle. Both exceptions to this general rule occurred in right vagi. In one case the threshold for the chronotropic effect on the homolateral auricle was lower than the inotropic effect; unfortunately the contralateral auricle was not studied in this animal. In the other exception, the thresholds were identical for the inotropic and chronotropic effects in the contralateral auricle. In only one animal of this series did the left vagus fail to produce a chronotropic effect.

DISCUSSION. The results show that in the majority of cases, with sufficient strength of stimulating current, the right vagus can stop the beat of the sinus. A negative inotropic action in the sinus and caval veins is often associated with this decrease of the rate of beat, but it must be noted that the cessation of the beat is not due to the decline of the sinus contractility to zero, for in many experiments it has been possible to show that the sinus will still contract when stimulated mechanically, although the pacemaker mechanism is under complete inhibition by the vagus.

The results of the stimulation of the left vagus on sinus rate are similar to those just mentioned, although the number of experiments in which the left could stop the heart was not so great; particularly in *Pseudemys elegans*. Examination of the left caval veins indicated that the contractility of those parts was strongly diminished during the stimulation of the left nerve.

Both vagi can influence sino-auricular conduction as is evidenced by a lengthening of the sino-auricular interval. This lengthening gradually gives way to partial block where the sinus beats at a regular rate though somewhat slower than normal, followed by every other beat of the auricles. Occasionally, it is possible to adjust the current strength of the stimulator so that complete block will take place. In one experiment with the left

vagus, it was found possible to produce complete block without any apparent decrease in rate of beat of the sinus. This single result is similar to those reported by von Skramlik (1932) in *Testudo graeca*, who concludes that this nerve does not innervate the pacemaker for the heart, since the left vagus could only produce slowing of the auricles and the ventricles by block at the sino-auricular junction and not by influencing the rate of the pacemaker. This may be the case in the species in question, but in our experiments on *Clemmys marmorata* it was the exception rather than the rule.

The results on the chronotropic and inotropic effects, as indicated by auricular contraction records, agree with those of Gilson (1930) and Heinbecker (1931) with two exceptions, one where the chronotropic effect appears to have a lower vagal threshold than the inotropic and one where the thresholds are identical. It must be noted that there is generally an overlap between these two effects. That is, beyond the chronotropic threshold, there is still greater reduction in auricular contractility or negative inotropic effect. Heinbecker also refers to this overlap, but concludes that in spite of such a fact, "it seems not inconsistent" to consider that the inotropic mechanism is innervated by a certain set of fibers and the chronotropic by an other type. However, this overlapping and the one exceptional case of a lower chronotropic threshold in the right vagus which we have found in our work are hard to reconcile with the concept of inotropic and chronotropic fiber types. In any event the question of the existence of distinctive inotropic and chronotropic fibers remains without definite answer.

CONCLUSIONS AND SUMMARY

The distribution of function of the right and left vagus nerves shows considerable variation in the two species of turtle examined. Such variations may have an important bearing on the interpretation of experimental results. Chronotropic and inotropic effects can be separated to a certain extent on the basis of stimulation thresholds of the vagus trunks but a definite overlapping exists. We have been unable to separate chronotropic and dromotropic effects on the basis of thresholds. It would thus seem that no definite evidence can be brought forward against the concept that a common mechanism is mediated by the vagus fibers and that the different effects produced are the result of the distribution of these fibers.

Our results, like those of Cohn (1912) on the dog, would appear to indicate that there exists a wide variation in the distribution of vagus fibers to the heart; this offers great difficulty in experimental work on the innervation of this organ. We may assume that the integrated control of this organ is mediated by numerous fibers producing chronotropic, inotropic, or

dromotropic effects according to the regions of the organs which they innervate, but the part played by these various effects in the economy and function of the organ remains obscure.

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STIMULATION OF THE HYPOTHALAMUS WITH SPECIAL REFERENCE TO ITS EFFECT ON GASTRO-INTESTINAL MOTILITY¹

H. KABAT, B. J. ANSON, H. W. MAGOUN AND S. W. RANSON

*From the Institute of Neurology and Department of Anatomy, Northwestern University
Medical School*

Received for publication February 20, 1935

A series of investigations is being conducted in this laboratory on the vegetative responses to electrical stimulation of the hypothalamus. The present report is concerned with the gastro-intestinal responses. Ranson and Magoun (1) reported increased rate and amplitude of respiration and marked dilatation of the pupils from stimulation of the lateral hypothalamic area and later work (2, 3) showed that a rise in blood pressure, contraction of the bladder and frequently struggling could be obtained from the same region. The above experiments were done under nembutal anesthesia, which is known to depress intestinal motility (4). To avoid such effects, the present experiments were conducted in the waking animal.

It should be emphasized that the hypothalamus is a very small region at the base of the diencephalon on either side of the third ventricle, comprising, in the cat, a cube each dimension of which is approximately 4 mm. There are a number of distinct nuclei, some having efferent connections with the posterior and intermediate lobes of the hypophysis, others sending impulses caudally to lower visceral centers. This region is very difficult to reach by ordinary methods. The difficulty has in great measure been overcome by the use of the Horsley-Clarke stereotaxic instrument to orient the electrodes. With the aid of this apparatus, it is possible to place the tip of the electrode within reasonable limits at any desired point in the interior of the brain and to stimulate a localized area of brain tissue immediately surrounding that point. The point is located by using the millimeter scales on the instrument, the coördinates being known from a study of serial sections of normal cat brains.

METHODS. Twenty-nine cats were used. The animal was anesthetized with ether, the calvarium exposed, and the Horsley-Clarke instrument adjusted to its head. The details of the construction of the instrument and of its use have been described by Horsley and Clarke (5) and Ranson (6).

¹ Aided by a grant from the Rockefeller Foundation.

A point on the surface of the skull was located by means of Horsley-Clarke coördinates and a small trephine hole was made. A metal cuff of the same diameter was screwed into the hole to the point where it was held firmly by the bone and exerted a slight tension on the dura below.

With the aid of the instrument, the electrode was put down to the desired point in the brain. The bipolar electrode was made of three lengths of enamelled nichrome wire cemented together, two fine wires (28 gauge) and a heavy wire (22 gauge) for support. Insulation covered all of the heavy wire and all of the fine wires except the tips of the latter, which were separated by about 1 mm. along the axis of the electrode. In three cats, special electrodes which had four fine wires cemented around a supporting wire were used, only the tips of the fine wires being uninsulated. The ends of two fine wires, separated by 1 mm., extended to the tip of the electrode, while the ends of the other two wires, also separated by a millimeter, only extended to within 8 to 10 mm. of the tip. With this type of electrode, two points, one more dorsally placed than the other, could be stimulated in the same cat.

In every case preliminary stimulation was conducted to determine from the response whether the electrode was at the desired point. From one to ten punctures were made, usually less than five, and several points were stimulated along each puncture. The method of exploration is described in detail by Ranson and Magoun (1). Once the electrode was properly oriented, it was left in place. An additional electrode was inserted in six of the cats, in one instance with the aid of the instrument, in others by hand; in three cats, the special electrodes described above were used, making possible the stimulation of two points in each brain.

Then the dura was carefully dried and dental cement was packed around the electrode and the cuff. When the cement was hard, the instrument was removed from the cat's head and the supporting wire cut level with the cement. The skin wounds were clipped and the animal was bound, lying on its side, in a comfortable hammock. After this the anesthesia was stopped. Not until two and one-half hours later, on the average, by which time the cat had fully recovered from the anesthesia, was the barium meal administered by stomach tube, and fluoroscopic observation of the alimentary tract begun.

All radiographic observations were made with a standard Victor 5 inch 30 M stabilized x-ray apparatus, supplied with a Coolidge 30 MA tube; a milliamperage of 6 was uniformly employed. During the progress of the observations several short exposures were made within the predetermined interval between peristaltic waves, in order that not even minor changes in the digestive tube would escape attention. At all times during exposure the outline of the barium meal was remarkably clear.

Observations were made of the peristaltic movements of the stomach

and intestine and the number of gastric waves initiated in one minute were counted. Faradic stimulation, of intensities varying from below threshold to strengths sufficient to give fully developed reactions, was then applied. The response of the gastro-intestinal tract was noted and latent period and after-effect were carefully recorded. To facilitate the observation of other responses to the stimulus, both vegetative and somatic, the lights were then turned on, preventing simultaneous visualization of the gut. This procedure was repeated a number of times, varying the coil distances and allowing time between stimuli for recovery.

After sufficient data had been collected, the animal was killed with ether, and in some cases the mucous membrane of stomach and small intestine was examined. The head was then injected with formalin and when the brain was hard, the bone was cut around the cuff and the electrode pulled out vertically with great care. The brain was removed and a block containing the region stimulated was embedded and cut into transverse sections, which were stained by Weil's method. The space that the electrode had occupied was easily identified and the point stimulated was located on a drawing of a transverse section of the brain stem.

RESULTS. Despite the operation, punctures and electrode in the brain, and frequent stimulation, gastro-intestinal movements between stimuli were as vigorous and as rapid as in normal controls. The cats would usually lie quietly, sometimes purring. In the animals which cried out and struggled occasionally, peristalsis was not disturbed. In two cats, however, one of which was a pregnant female, no peristalsis was seen during the entire period of observation. The electrode in both cases pierced the infundibular stalk, one lodging in the anterior lobe of the pituitary gland. The experimental animals were as variable as normal ones in regard to the time between feeding and the first appearance of peristalsis or the first passage of meal through the pyloric sphincter, the site of the electrode apparently not being a factor.

The points stimulated have been classified according to location and will be discussed in the following order:

- A. Hypothalamus—8 points.
- B. Very close to hypothalamus—2 points.
- C. Septum pellucidum, preoptic area and anterior commissure—11 points.
- D. Internal capsule and basis pedunculi—6 points.
- E. Infundibular stalk and its environs—6 points.
- F. Two points stimulated in the same brain—9 cats.

A. *Hypothalamus.* The response of the gastro-intestinal tract to stimulation of the hypothalamus was an immediate inhibition of peristalsis and complete loss of tone. The latent period for abolition of gastric waves was very short, approximately one second. If stimulation was begun when a peristaltic wave was halfway along the stomach wall, the wave

stopped abruptly and never reached the sphincter. If the wave was very close to the sphincter when stimulation was initiated, some meal was ejected into the duodenum, but instead of passing rapidly through the latter as is normally the case, the small mass remained stationary at its upper end. The latent period for cessation of intestinal movements was equally short. Loss of tone came on a little more slowly, the stomach losing its constrictions and becoming baglike, the intestine losing its beading and becoming dilated and ribbon-like. The pyloric sphincter may have been contracted, for the rapid, deep respiration and frequent violent struggling which occurred during stimulation could probably raise intra-gastric pressure sufficiently to discharge some meal through a relaxed sphincter. The inhibition was maintained during stimulation and for a variable period, usually about 30 to 90 seconds, after termination of the stimulus. This after-inhibition may be due to sympathetic after-discharge or to a secretion of adrenalin.

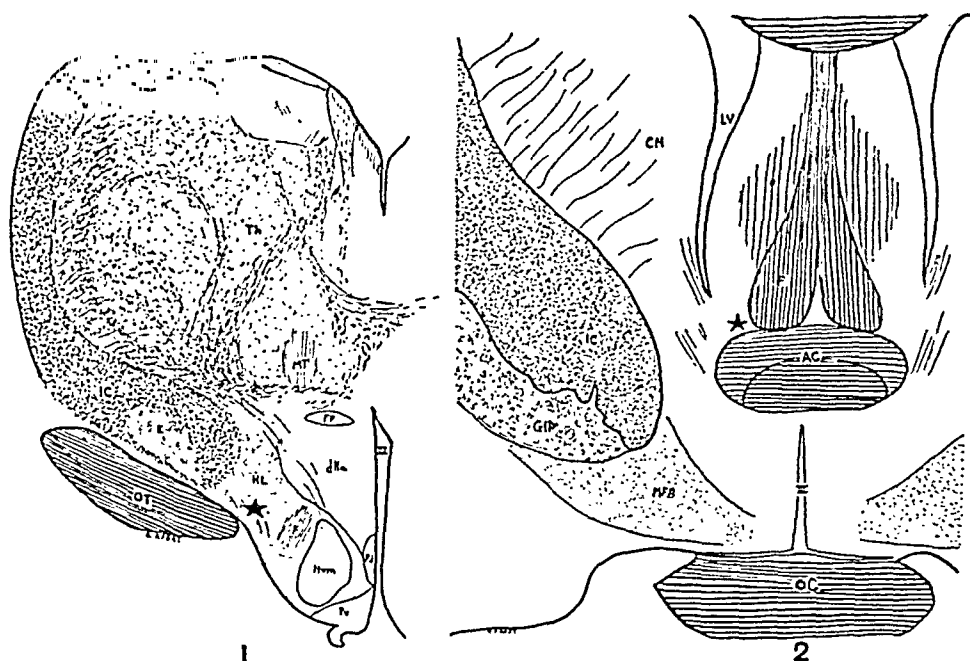
Accompanying the gastro-intestinal inhibition were marked dilatation of the pupils, greatly increased rate and amplitude of respiration, and erection of hair.² Frequently vigorous struggling occurred. Sometimes the head was raised when stimulation was begun and the cat looked from side to side excitedly and very often the claws were protruded, the eyes opened wide and the nictitating membranes retracted. Urination occurred during stimulation in several experiments and in one case rhythmic spitting was observed.

Microscopical examination of the brains showed that in eight of the cats, the tip of the electrode was in the hypothalamus (fig. 1). The exact points stimulated and the threshold coil distances for inhibition of peristalsis were:

- Cat 1—in lateral hypothalamic area, 1 mm. from base, lateral to rostral end of mammillary body. Inhibition at coil 20.
- Cat 2—in lateral hypothalamic area, $2\frac{1}{2}$ mm. from base, lateral to rostral part of nucleus hypothalamicus posterior. Inhibition at coil 12.
- Cat 3—in lateral hypothalamic area, 1 mm. from base, lateral to caudal part of nucleus hypothalamicus posterior. Inhibition at coil 15.
- Cat 5—in lateral hypothalamic area, $\frac{1}{2}$ mm. from base, lateral to caudal part of nucleus hypothalamicus ventromedialis. Inhibition at coil 14 (fig. 1).
- Cat 6—at junction of lateral hypothalamic area and field H_2 of Forel, $1\frac{1}{2}$ mm. from base, lateral to middle of mammillary body. Inhibition at coil 15.
- Cat 21—in rostral part of nucleus hypothalamicus ventromedialis, 2 mm. from base, 1 mm. medial to fornix. Inhibition at coil 14.
- Cat 26—in lateral hypothalamic area, 1 mm. from base, lateral to middle of mammillary body. Inhibition at coil 14.
- Cat 29—in supramammillary commissure in midline, dorsal to middle of mammillary body. Inhibition at coil 15.

² This group of responses will henceforth be designated, for want of a better term, as "sympathetic discharge," although the respiratory movements are performed by skeletal muscles innervated by somatic nerves.

All of these points were in the lateral hypothalamic area except two, one of which was in the supramammillary commissure, the other in the nucleus hypothalamicus ventromedialis. The fact that inhibition was obtained from stimulation of the supramammillary commissure may be explained by unpublished observations indicating that impulses from the lateral hypothalamic area cross in part in this commissure. There is a possibility that the inhibition obtained from stimulation of the ventromedial nucleus may not have been due to stimulation of this nucleus but to the spread of current to the lateral hypothalamic area. The assumption that such spread



Stars represent the exact points stimulated.

Fig. 1. Transverse section through the diencephalon of the cat at about the middle of the hypothalamus. Stimulation of the lateral hypothalamic area.

Fig. 2. Transverse section through the telencephalon at the level of the crossing of the anterior commissure. Stimulation of the dorsal surface of the anterior commissure.

occurred is borne out by the observation that inhibition was never obtained from this nucleus unaccompanied by rhythmic spitting. The latter response is localized in the rostral part of the lateral hypothalamic area, in the supraoptic region, about $\frac{1}{2}$ mm. from the point in the ventromedial nucleus.

If the coil distance was too great to produce cessation of peristalsis, stimulation had no effect on tone or motility, but did, however, result in a moderate sympathetic discharge. At the threshold for the gastro-intestinal response, the inhibition was irregular. Sometimes under these conditions peristalsis would stop on applying the stimulus but would appear

again at the normal rate before stimulation was ended. Occasionally no inhibition occurred until some time after the beginning of stimulation. Furthermore, a threshold stimulus that was effective in stopping peristalsis was often ineffective on repetition.

The relation of struggling to the inhibition is of interest. Spontaneous struggling occurred frequently in several animals with no inhibition of peristalsis. During stimulation of the hypothalamus, struggling was sometimes observed without inhibition. When the cat responded to the stimulus with violent struggling, together with a marked sympathetic discharge, cessation of peristalsis always supervened; but the struggling was not essential to the inhibition, since definite inhibition has been observed with no struggling at all and inhibition was often evident long before the onset of struggling.

In some of these cats, the mucous membrane of stomach and small intestine was examined and no hyperemia, hemorrhage or ulceration was seen.

B. Points close to hypothalamus. Cessation of peristalsis was observed in two other cats at coil distances very similar to those that were threshold for the hypothalamus. The tips of the electrodes were found, when the sections were studied, very close to the lateral hypothalamic area:

Cat 4—at base of brain, 1 mm. lateral to mammillary peduncle, about $\frac{1}{2}$ mm. caudal to mammillary body. Inhibition at coil 13.

Cat 27—in field H_1 of Forel of subthalamus, 3 mm. from base, 2 mm. from midline, at level of caudal part of mammillary body. Inhibition at coil 15.

The sympathetic discharge accompanying the inhibition in cat 4 was similar to that obtained from stimulation of the lateral hypothalamic area. Stimulation of the subthalamus, besides eliciting a sympathetic discharge, caused extension of the ipsilateral limbs and flexion of the contralateral limbs.

C. Septum pellucidum, preoptic area and anterior commissure. As a control measure, eleven points were stimulated in the septum pellucidum, preoptic area and anterior commissure in nine cats (fig. 2). No change in peristalsis was observed on stimulation of these regions, using stimuli of a strength that would yield immediate inhibition from the lateral hypothalamic area. Careful study revealed no change in the rate of gastric peristalsis or in the tone of the stomach and intestine. Stimulation was frequently effective in producing urination, slight dilatation of the pupils, slowing or slight acceleration of respiration and in some cases rhythmic crying. The lack of excitement and struggling formed a striking contrast to the vigor of the hypothalamic response.

No gastro-intestinal response was observed, as the secondary coil was approximated to the primary until there was evidence of spread of current

to the hypothalamus when cessation of peristalsis supervened. A marked sympathetic discharge and in three cases also crying accompanied the inhibition of the gut. In one cat stimulated in the septum, a more moderate response accompanied the inhibition—the respiration was greatly accelerated but there was no struggling or erection of hair and only slight dilatation of the pupils.

The coils had to be much closer together for inhibition of peristalsis to result from stimulation of the septum, etc., than from stimulation of the lateral hypothalamus. The characteristics of the inhibition, the latent period and after-inhibition were the same as have been described for the latter region. The location of the various points and the threshold coil distances for inhibition follow:

- Cat 7—in medial preoptic area in midline, $\frac{3}{4}$ mm. rostral to crossing of anterior commissure. Inhibition at coil 10.
- Cat 8—in septum pellucidum, $\frac{3}{4}$ mm. rostral to crossing of anterior commissure near tip of lateral ventricle. Inhibition at coil 7.
- Cat 8—(second electrode)—in anterior commissure at its crossing, 2 mm. from midline. Inhibition at coil 4.
- Cat 11—(second electrode)—at base of brain, 1 mm. from midline, at level of crossing of anterior commissure. Inhibition at coil 9.
- Cat 15—in medial preoptic area in midline, $\frac{3}{4}$ mm. rostral to crossing of anterior commissure. Inhibition at coil 10.
- Cat 15—(second electrode)—at base of brain, 5 mm. from midline, $\frac{3}{4}$ mm. rostral to crossing of anterior commissure. Inhibition at coil 9.
- Cat 16—On dorsal surface of anterior commissure at level of its crossing, $1\frac{1}{2}$ mm. from midline. Inhibition at coil $9\frac{1}{2}$ (fig. 2).
- Cat 17—in bed-nucleus of anterior commissure, $\frac{3}{4}$ mm. rostral to the commissure. Inhibition at coil $12\frac{1}{2}$.
- Cat 18—in bed-nucleus of anterior commissure, $\frac{3}{4}$ mm. rostral to the commissure. Inhibition at coil $11\frac{1}{2}$.
- Cat 19—in septum pellucidum in midline, $1\frac{1}{2}$ mm. rostral to crossing of anterior commissure. Inhibition at coil 9.
- Cat 20—in interbulbar component of anterior commissure, at its crossing, 1 mm. from midline. Inhibition at coil 9.

D. Internal capsule and basis pedunculi. In five cats, the corticofugal motor fibers were stimulated. The tip of the electrode was found, on examination of the brains, in the internal capsule rostral to the hypothalamus in four cases (fig. 3), while in the other it was located in the midbrain, at the lateral edge of the basis pedunculi. Stimulation of these points, using coil distances that would evoke inhibition from the lateral hypothalamic area, had no effect on peristalsis. These stimuli did result in moderate dilatation of the pupils, some acceleration of respiration, rhythmic lapping and movements of the contralateral forelimb. As the secondary coil was moved closer to the primary, movements of other muscle groups were added to the list. At the threshold for inhibition of the

digestive tract, a sympathetic discharge occurred besides vigorous chewing or lapping, flexion of contralateral and extension of ipsilateral limbs, contraction of neck muscles and occasionally of facial muscles. The inhibition was the same as in the preceding groups of experiments, except in cat 13, in which inhibition did not set in until two shallow waves were completed. No data were collected on after-inhibition. A peculiar phenomenon was recorded in cat 14. In this animal, inhibition of the stomach occurred three times on stimulation at coil 12. The intestine was inhibited only at first, for movements resumed during stimulation. Later stimulation at coil 12, $11\frac{1}{2}$, 11 and 10 was ineffective in stopping peristalsis in the

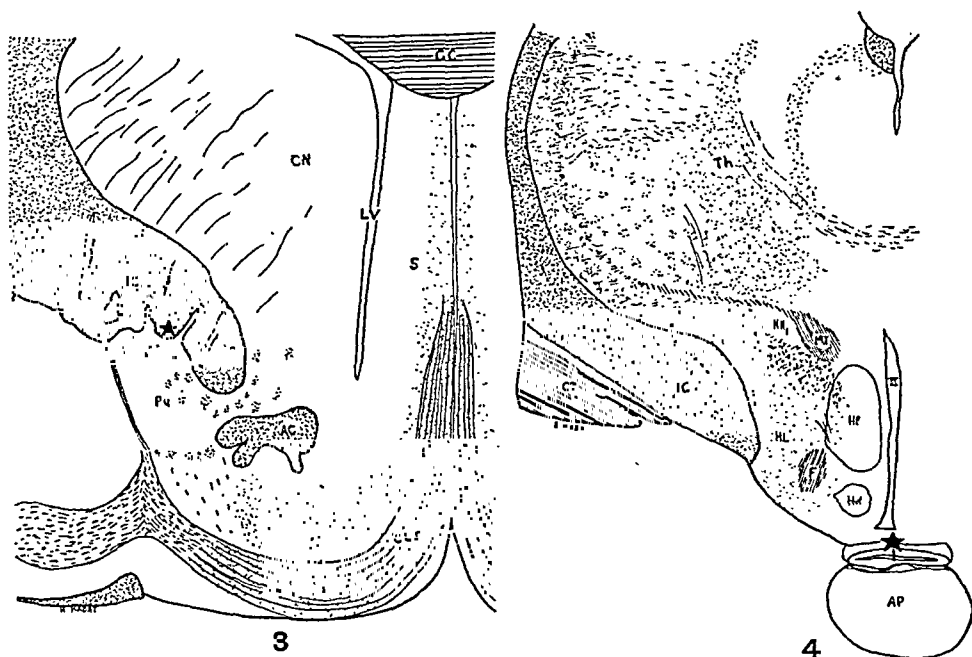


Fig. 3. Transverse section through the telencephalon in front of the crossing of the anterior commissure. Stimulation of the internal capsule.

Fig. 4. Transverse section through the diencephalon at about the middle of the hypothalamus. Stimulation of the infundibular stalk.

stomach or intestine and complete inhibition did not appear until stimulation was carried out at coil $9\frac{1}{2}$.

The exact location of the points stimulated and the threshold for inhibition were as follows:

- Cat 10—at lateral edge of basis pedunculi, about 1 mm. caudal to mammillary body. Inhibition at coil 10.
- Cat 10—(second electrode)—in internal capsule dorsal to lateral geniculate body. No inhibition at coil 0.
- Cat 11—at ventral edge of internal capsule, 2 mm. from its medial border, $\frac{3}{4}$ mm. rostral to anterior hypothalamic area. Inhibition at coil 10.

- Cat 12—in internal capsule, 1 mm. from its medial border, among anterior commissure fibers traversing the internal capsule. Inhibition at coil 6.
- Cat 13—in external capsule $\frac{1}{2}$ mm. ventrolateral to internal capsule at a level $2\frac{1}{2}$ mm. rostral to crossing of anterior commissure. Inhibition at coil 8.
- Cat 14—in ventral part of internal capsule, 1 mm. from its medial border, 2 mm. rostral to crossing of anterior commissure. Inhibition at coil 12 and $9\frac{1}{2}$ (fig. 3).

E. Infundibular stalk. An attempt was made in six cats to stimulate the infundibular stalk, in order to activate the hypothalamico-hypophyseal mechanism but in only two cases was the electrode properly placed for this purpose (fig. 4). Stimulation of the stalk in these two animals and of points near the stalk in two others using coil distances effective in stopping peristalsis on stimulation of the lateral hypothalamic area, produced no change in motility or tone of the alimentary tract; no increase or decrease in activity was ever observed. Such stimulation did, however, cause a slight dilatation of the pupils, a slight acceleration of respiration; and in one case urination, but the animal remained quiet, showing no signs of excitement. After numerous stimuli, gastric peristalsis, as measured by the number of waves initiated per minute, was no more marked than at the beginning of the experiment. When the current was increased sufficiently in cats 22, 23, and 25, stimulation of the stalk resulted in cessation of peristalsis, along with vigorous spitting and crying, urination, and a marked sympathetic discharge. In cat 14, the same response occurred without spitting or crying. Somewhat more moderate sympathetic and somatic discharges took place on stimulation at coil distances too great to inhibit the gut. The fact that in two cats with electrodes in this region there was no peristalsis at all seen during the experiment has already been mentioned.

The gastric and intestinal mucous membrane in each of these animals was examined at the end of the experiment and no hyperemia, hemorrhage, or ulceration were discovered.

A microscopical study of the brain revealed that the tip of the electrode was successfully oriented in two of the cats. The points stimulated and the thresholds for inhibition were as follows:

- Cat 14—(second electrode)—out of brain, about $\frac{1}{2}$ mm. ventrolateral to middle of mammillary body. Inhibition at coil 10.
- Cat 22—at base of brain, $\frac{1}{2}$ mm. lateral to infundibular stalk, at level of rostral part of nucleus hypothalamicus posterior. Inhibition at coil $9\frac{1}{2}$.
- Cat 23—out of brain, about $\frac{3}{4}$ mm. ventral to infundibular stalk in midline, in front of pituitary. Inhibition at coil $9\frac{1}{2}$.
- Cat 24—in anterior lobe of pituitary. No peristalsis seen.
- Cat 25—in infundibular stalk in midline at level of posterior hypothalamic nucleus. Inhibition at coil 11 (fig. 4).
- Cat 28—through infundibular stalk in midline, in front of pituitary. No peristalsis seen.

F. Two points stimulated in the same brain. In nine cats of the series, two points were stimulated in the same brain, making possible the comparison of thresholds for inhibition for different regions with the disturbing factor of individual variability eliminated. For example, in cat 10, stimulation of the basis pedunculi resulted in inhibition when the coil was at 10 cm. Stimulation of another point in the internal capsule dorsal to the lateral geniculate body elicited no change in peristalsis even when the secondary coil was at 0. In cat 15, two points in the preoptic area had thresholds of 10 and 9 cm. In cat 14, the electrode in the internal capsule and the one in the infundibular stalk had about the same threshold. In cat 9, inhibition was obtained on hypothalamic stimulation at coil 17, while coil 10 was the threshold for a point more dorsally placed. The results indicate that the hypothalamus has the lowest threshold and that the coil distance required for inhibition is similar for internal capsule, preoptic area and infundibulum.

The most convincing of this group of experiments were the three in which two points were stimulated along a single electrode, one in the hypothalamus and one in the thalamus (fig. 5). The location of the points and the thresholds were:

- Cat 21—(a) in nucleus hypothalamicus ventromedialis. Inhibition at coil 14.
(b) on surface of anterodorsal nucleus of thalamus. Inhibition at coil 11½.
- Cat 26—(a) in lateral hypothalamic area at level of mammillary body. Inhibition at coil 14. (b) in nucleus lateralis pars posterior of thalamus, 2½ mm. from midline. Inhibition at coil 9 (fig. 5).
- Cat 27—(a) in field H₁ of Forel. Inhibition at coil 15. (b) about 1 mm. dorsal to surface of thalamus, in region of pulvinar. Inhibition at coil 9.

The vigorous responses to stimulation with the hypothalamic electrodes have been adequately described. Stimulation of the thalamus, using coil distances of 15 to 12 cm. caused no change in peristalsis and only occasional slight dilatation of the pupils. Stronger stimulation of the thalamus that resulted in inhibition of the gut also produced marked dilatation of the pupils and rapid, very shallow respiration, followed after 20 to 30 seconds by a very violent clonic convulsion that lasted for a minute or two and was accompanied by profuse salivation. The fit proceeded whether the stimulation was continued or not. If the current was strong enough, the stimulus could be stopped after 10 seconds and the convulsions would follow. This mechanism was easily fatigued and would only repeat at reduced coil distances. As a rule, the gastro-intestinal inhibition began with the onset of the fit and not when stimulation was started. It lasted during the convulsions and also for a minute or two afterward, a rather brief after-inhibition following such a violent reaction. In cat 21, inhibition occurred without a fit.

Convulsions have also been observed in eight cats during very strong stimulation of the hypothalamus (secondary coil set at 4 cm.). These convulsions differed from those elicited from the thalamus in being much less clonic in character, in coming on at once at the beginning of stimulation and in stopping immediately on removal of the stimulus. It should be emphasized, however, that nothing resembling a convulsion ever occurred

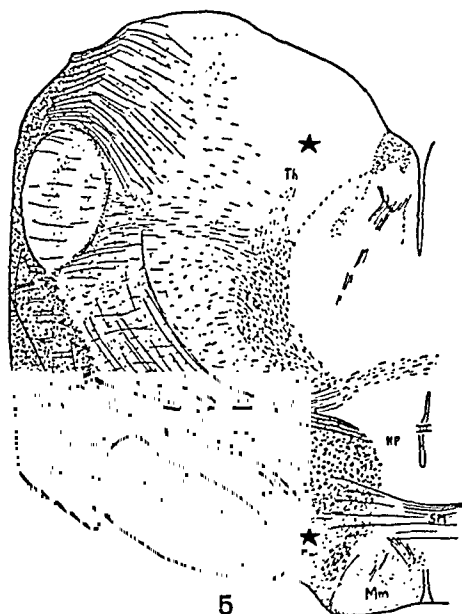


Fig. 5. Transverse section through the diencephalon at the level of the mammillary bodies. Stimulation of two points, one in the lateral hypothalamic area and one in the thalamus.

List of abbreviations: *AC*, anterior commissure; *AP*, anterior pituitary; *BP*, basis pedunculi; *CC*, corpus callosum; *CN*, caudate nucleus; *dHa*, dorsal hypothalamic area; *E*, entopeduncular nucleus; *F*, fornix; *FP*, nucleus filiformis principalis; *GIP*, globus pallidus; *HL*, lateral hypothalamic area; *HP*, nucleus hypothalamicus posterior; *Hvl*, nucleus hypothalamicus ventrolateralis; *Hvm*, nucleus hypothalamicus ventromedialis; *H₁*, *H₁* field of Forel; *I*, infundibular stalk; *IC*, internal capsule; *LV*, lateral ventricle; *MFB*, medial forebrain bundle; *Mm*, medial mammillary nucleus; *MT*, mammillo-thalamic tract; *NH₁*, nucleus of *H₁* field of Forel; *OC*, optic chiasma; *OLS*, olfacto-septal fibers; *OT*, optic tract; *Pd*, nucleus hypothalamicus periventricularis dorsalis; *Pu*, putamen; *Pv*, nucleus hypothalamicus periventricularis ventralis; *S*, septum pellucidum; *SM*, supramammillary commissure; *STh*, subthalamic nucleus of Luys; *Th*, thalamus; *III*, third ventricle.

with the strengths of current used in eliciting from the hypothalamus an inhibition of the gastro-intestinal musculature and the other sympathetic and somatic responses described in this paper.

A number of observations have accumulated on the effects of pain on gastro-intestinal motility. Pinching of the paw caused struggling, crying, rapid respiration, dilatation of the pupils and sometimes erection of hair.

It had no effect on peristalsis in two out of three normal animals and in nine out of eleven operated animals studied.

DISCUSSION. In recent years, the brain has been stimulated in unanesthetized animals by Pachon and Delmas-Marsalet (7), Hess (8) and Mussen (9).

The use of the x-ray after a barium meal to study gastro-intestinal motility is the most satisfactory, since no operative procedure on the abdomen is required and changes in tone and motility can be clearly seen.

Our results indicate that there is a center in the hypothalamus which inhibits gastro-intestinal motility. Just how much of the hypothalamus is concerned in this inhibitory mechanism we cannot say for certain. The lateral hypothalamic area is surely involved, since stimulation of this region always results in gastro-intestinal inhibition. Furthermore, it is known that the responses of the pupils, the bladder, blood pressure and respiration are most marked on stimulation of this region (1, 2, 3) and since no inhibition occurs unaccompanied by a marked sympathetic discharge, it is safe to assume that the single center is concerned in all of these responses. The infundibulum may be eliminated from consideration on the basis of the negative results of the two experiments described above. The possibility, however, that the medial hypothalamus is part of the inhibitory center cannot be excluded, since the only point stimulated in this region (the ventromedial hypothalamic nucleus in cat 21) did produce abolition of digestive motility. Stimulation of the supramammillary commissure in one experiment caused inhibition, suggesting that these fibers are part of the pathway from the hypothalamus to the gut.

All components of the response to stimulation of the hypothalamus do not have the same threshold and the gastro-intestinal inhibition seems to have the highest of any. Dilatation of the pupils, erection of hair, increases in rate and depth of respiration, urination, and struggling have resulted from stimulation at coil distances too great to yield cessation of peristalsis.

Observations on the effects of subcortical stimulation on the motility of the alimentary tract have been made by some other investigators (10, 11, 12, 13, 14, 15) with results differing considerably from our own.

One by one, the changes that Cannon (16) has observed in his emotionally excited cats are being found to follow electrical stimulation of the hypothalamus. Karplus and Kreidl, beginning in 1909, investigated these responses. In a summary of their work, Karplus (17) lists pupillary dilatation, rise in blood pressure, contraction of the bladder, secretion of sweat, tears and saliva, and occasional crying as responses to hypothalamic stimulation. Recently their findings have been confirmed and more precise information on localization obtained (1, 2, 3). In the present paper, with waking animals, we have added erection of hair, the intense expression and violent movements typical of strong emotional reactions,

even including snarling and baring of the claws as well as inhibition of gastro-intestinal motility to the growing list. The similarity between the response of a cat to a barking dog and its response to faradic stimulation of the lateral hypothalamic area is now perfectly clear. This is in full agreement with the conclusions reached by Bard (18) and points to the hypothalamus as the center responsible for the integration of the reaction pattern characteristic of intense emotional excitement (19).

CONCLUSIONS

1. Electrical stimulation of the lateral hypothalamic area in the unanesthetized cat causes cessation of peristalsis and loss of tone of the stomach and small intestine.

2. Stimulation of the thalamus, internal capsule, anterior commissure, septum pellucidum and infundibular stalk with the same strength of current produces no change in gastro-intestinal motility.

3. No increases in tone or motility were observed at any time from any of the various points stimulated in the brain.

4. These results are discussed in relation to the hypothesis that the hypothalamus is the integrating center for emotional reactions.

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THE AMERICAN JOURNAL OF PHYSIOLOGY

VOL. 112

JUNE 1, 1935

No. 2

RESPONSE OF THE HYPERTHYROID HEART TO EPINEPHRINE

C. H. McDONALD, WALTER LEE SHEPEARD, M. F. GREEN
AND A. F. DEGROAT

From the Department of Physiology and Pharmacology, University of Arkansas, School of Medicine, Little Rock

Received for publication February 27, 1935

The effect of thyroid substance or of thyroxine upon tissues is the subject of much investigation the results of which are not always in agreement. Kalnins (1928) reported no increase in the effect of epinephrine upon the excised frog heart following perfusion with a solution containing thyroxine; he reported a slightly lower heart rate with increased amplitude of beat following such perfusion. Paasch and Reinwein (1928), Hopping (1931) and others found no increase in tissue metabolism from thyroxine. Davis, Da Costa and Hastings (1934) have recently demonstrated a marked but delayed increase in the metabolism of the isolated frog heart on perfusion with thyroxine. Their work does not reveal whether this increased metabolism is due to an increased beat of the heart.

Prolonged administration of thyroid has generally shown an increased activity of structures innervated by the sympathetic system. A tachycardia (MacIntyre, 1931), an augmented response to epinephrine by the iris (Bergwall and Kuchinsky, 1931), an augmented blood pressure and heart rate (Santesson, 1919; Lütolf, 1930), and a restoration to normal metabolism, heart rate, and response to epinephrine in thyroidectomized cats (Sawyer and Brown, 1935) are described following administration of thyroid or thyroxine. It is theorized that thyroid sensitizes the structures upon which epinephrine acts.

This series was designed to study simultaneously the rate of beat, the rate of oxygen consumption, and the response to epinephrine of the isolated heart of terrapins made hyperthyroid by the prolonged administration of desiccated thyroid perorally. The apparatus employed and the manner of perfusion were described by one of us (McDonald and McDonald, 1935).

A suspension of desiccated thyroid substance¹ in water was placed in a small hypodermic syringe fitted with a no. 13 Fr. catheter. The terrapin's head was withdrawn from its shell, its jaws held open with a screw clamp, the catheter introduced as a stomach tube, and sufficient of the suspension injected to equal 0.001 gram for each 10 grams of body weight as the initial dose; later dosage was 0.001 gram for each 50 grams of body weight. The animals were given thyroid substance once each week for a period of four weeks. There was an early loss of weight, a diarrhea with yellowish, mucus-laden stools. A muscular weakness appeared after two or three weeks; if this weakness was marked or developed earlier than the average the dosage was cut down or omitted for a week. We lost four terrapins by death.

A pronounced tachycardia was accepted as evidence of a hyperthyroid state. Only 1 of 18 terrapins to which thyroid was administered failed to respond with a heart rate much more rapid than that exhibited by the controls. This heart was discarded as were those of two controls which exhibited rates substantially more rapid than that which we have observed to be average for the isolated terrapin heart. The perfusion fluid in all experiments was of the following composition:

	<i>per cent</i>
Sodium chloride.....	0.650
Potassium chloride.....	0.014
Calcium chloride.....	0.012
Sodium bicarbonate.....	0.020
Sodium dihydrogen phosphate.....	0.001

Observations were for periods of one hour unless otherwise indicated; the hearts were then laid open, blotted dry, and weighed. The oxygen consumed was reduced to standard conditions and calculated in cc./gm./hr. Table 1 sets forth, in averages, the rate of oxygen consumption and of beat of the isolated hearts of controls and of terrapins made hyperthyroid through prolonged peroral administration of thyroid.

Table 2 sets forth, in averages, the rate of oxygen consumption, rate of beat, and response to epinephrine of control and hyperthyroid hearts. These were perfused with the Ringer's solution for 30 minutes, observations being made upon the rate of oxygen consumption and rate of beat; epinephrine-HCl solution was then added to the perfusing fluid to the concentration of 1:500,000. The point of introduction of the epinephrine into the apparatus delayed for a short time a response by the heart. Maximum increase in the rate of beat was observed to occur at an average of 3 minutes following introduction of the epinephrine; the rate at this time was selected

¹ The desiccated thyroid substance was furnished through the courtesy of Parke, Davis & Co.

as the standard response in rate of beat to epinephrine. The oxygen consumption was determined for a period of 30 minutes following the introduction of epinephrine.

Acting upon the theory that the tachycardia of hyperthyroidism is due to a sensitization of the structures upon which epinephrine acts, we attempted to paralyze the sympathetic endings in the isolated heart through

TABLE 1

A comparison of the rate of oxygen consumption and the rate of beat of control terrapins and hyperthyroid terrapins

TYPE	NUMBER OF EXPERIMENT	O ₂ CONSUMPTION	HEART RATE		INCREASE O ₂ CONSUMPTION	RATE OF BEAT
			Initial	Final		
		cc./gm./hr.			per cent	
Control.....	6	1.57	27	25		
Hyperthyroid.....	10	2.76	44	40	75	63

TABLE 2

A comparison of the response to epinephrine by the isolated hearts of control terrapins and hyperthyroid terrapins

TYPE	NUMBER OF EXPERIMENT	O ₂ CONSUMPTION		HEART RATE		INCREASE IN RATE FOLLOWING EPINEPHRINE	
		Ring.	Epin.	Ring.	Epin.	O ₂ consumption	Beat
		cc./gm./hr.				per cent	
Control.....	6	1.60	2.33	32	38	46	18.7
Hyperthyroid.....	6	3.08	6.98	46	56	126	21.7

TABLE 3

A comparison of the effect of ergotoxine upon the isolated hearts of control and of hyperthyroid terrapins

TYPE	NUMBER OF EXPERIMENT	HEART RATE			
		Initial → Ergotoxine → Epineph. → Ergotoxine			
Control.....	24	34	31	40	36
Hyperthyroid.....	2	48	34.5	46	48

the use of ergotoxine ethanesulphonate;² 0.5 gram of the substance was dissolved in 150 cc. of the perfusion fluid and recirculated through the heart several times. Alterations in the rate of contraction and in the response to epinephrine of these hearts are shown in table 3. Because of

² Ergotoxine ethanesulphonate furnished through courtesy of Eli Lilly & Co. and Abbott Laboratories.

reduction in the surface tension with slight frothing of the circulating medium, oxygen consumption observations were invalidated.

DISCUSSION. It is obvious from these experiments that prolonged feeding of thyroid to terrapins results in a tachycardia and an increased consumption of oxygen by the heart. The rate of increase in oxygen consumption parallels the rate of increase in heart beat close enough that when the factor of experimental error is taken into account there appears to be little, if any, increase in amplitude of beat as a result of prolonged administration of thyroid. In the response to epinephrine the hyperthyroid hearts showed a slightly greater increase in rate of beat and a much greater increase in rate of oxygen consumption than did the controls. The greater increase in amplitude and force of contraction in the hyperthyroid hearts following epinephrine is easily observable; whether these factors wholly account for the increased rate of oxygen consumption is not determined by these experiments.

SUMMARY

1. Prolonged feeding of thyroid to terrapins results in a tachycardia and an increased rate of oxygen consumption by the isolated heart.
2. It leads to an increased response to epinephrine; the increase in rate of beat is slight, that in rate of oxygen consumption is enormous as compared to the same response by controls.
3. If ergotoxine exerts any paralyzing effect upon the sympathetic structures within the heart in the dosage employed in these experiments, this effect is abolished by the action of epinephrine, both in the control heart and in the hyperthyroid heart.

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A STUDY IN REFLEXES: IDENTIFICATION OF THE CUTANEOUS AFFERENT FIBERS WHICH EVOKE IPSILATERAL EXTENSOR AND FLEXOR REFLEXES

A. SIDNEY HARRIS

*From the Physiological Laboratory of Washington University School of Medicine,
Saint Louis*

Received for publication January 28, 1935

The application of the cathode ray oscillograph to the study of nerve action potentials has in recent years provided criteria whereby the active fibers in a nerve may be differentiated in terms of fiber diameter. These criteria are 1, electrical threshold, and 2, rate of conduction of the impulse (Erlanger and Gasser, 1924; Gasser and Erlanger, 1927). By applying these criteria, Heinbecker, Bishop and O'Leary (1933, 1934) demonstrated a specificity of groups of afferent fibers for the skin senses: touch, pain, warmth and cold, and recognized the band that each occupies in the fiber spectrum. The employment of the above mentioned criteria in this study has demonstrated that qualitatively different reflex responses are evoked by relatively discrete groups of afferent fibers as characterized by diameter, irritability, and conduction rate, but there is overlapping of the ranges that they occupy.

In the early experiments, exploratory trial stimulation of various large nerves and posterior roots of the spinal bullfrog (*Rana catesbiana*), while registering the reflex contractions in ipsilateral extensor and flexor muscles of the hind limb, yielded variable and confusing results. The variations were similar to those reported by earlier workers as, for example, Graham Brown (1911), and Sherrington and Sowton (1911a and b). Usually the lowest threshold response was that of ipsilateral extension, sometimes flexion, and at other times both would appear simultaneously, or one quickly after the other without alteration of the applied stimuli. It appeared quite impossible to work out from the results of stimulation of large mixed nerves any system of differentiation by thresholds which would relate the type of response to any limited group of afferent fibers.

Further exploration revealed that stimulation of the skin posterior to the gastrocnemius muscle gave in many cases a reflex of apparently pure ipsilateral extension. Search of the literature then showed that not only did stimulation of this area yield primarily extension reflexes, but the same was also true of the skin in the region of the Achilles tendon, the plantar

surface, and over the triceps femoris muscle. The stimulation of certain other areas results wholly or chiefly in flexion. This is true of the skin of the toes, the dorsum of the foot, and the front of the leg. Light touch constitutes an adequate stimulus for either of these responses when applied to an appropriate area. Figure 2 is a record of extensor and flexor reflex contractions recorded from the triceps and semitendinosus muscles respectively, showing the specificity of localization of the areas from which the responses may be obtained. The stimulus used in this experiment was light stroking of the skin with a wet camel's hair brush. Light pinching with forceps, or weak electrical stimulation of the skin gives similar results.

None of the authors (Beritoff, 1913, 1923; Fröhlich, 1909; and Baglioni, 1904) who have reported on the reflexes evoked by stimulation of different skin areas dissected the nerves free from the skin and stimulated them directly, nor did they study their fiber composition. The cutaneous nerves of the leg and foot of the bullfrog were used in the experiments to follow.

METHODS. *Method of comparing the thresholds of the most irritable fibers in different nerves.* In order to be able to correlate reflex thresholds, found under conditions to be described later, with nerve fibers of any given irritability group it was necessary to have for comparison some standard of reference. As the most dependable and available standard the threshold of the most irritable motor fibers in some suitable nerve, as indicated by the contraction of the muscles innervated by the nerve, was chosen. Sherrington (1894) showed that the largest fibers in the nerves of the hind limb of the mammal are motor. Erlanger et al. (1926, 1927) demonstrated with the cathode ray oscillograph that the motor fibers are among the most irritable and most rapidly conducting fibers in the hind limb nerves of both the dog and frog. No differences in irritability or conduction rate were found between the most irritable motor and the most irritable sensory fibers.

When the reflex responses occurring upon stimulation of a cutaneous nerve are being studied, the same nerve obviously cannot be used as a part of a nerve-muscle preparation for comparison. Since it was necessary, therefore, to compare with another nerve it was essential to find a system by which it would be possible to make valid comparisons of the threshold of fibers in one nerve with that of fibers in another. There are two factors which qualify comparison, *a*, secondary resistance when stimulating with induced currents, and *b*, differences in the amount of shunting by inactive tissue (when stimulating by any method). Both differences are reduced relatively by an external shunt. For this purpose, use was made of a low resistance potential divider which was also employed to grade the stimulating voltage applied to the nerve. When this system was used the changes in threshold when the point of stimulation was moved from a large part to a small part of the nerve were no larger than the random variations at a point, namely, 2 to 4 per cent.

In the early experiments stimulation was accomplished by an inductorium operated by a rotating interrupter in the primary circuit. With high frequencies, however, this method is not entirely satisfactory due to possible chattering of keys and to foreshortening of the make shock which is believed to be short circuited (Erlanger and Garrey, 1914). Therefore a thyatron stimulator (see Schmitt and Schmitt, 1932) was employed in all high frequency experiments (60 to 120 per second, and sometimes higher). This stimulator is free from both of the difficulties which beset the other type of apparatus as it does not involve the use of any keys or short circuits.

In almost all of the experiments from which threshold data are reported, contact with the nerve was achieved through mercury-calomel non-polarizable electrodes. The glass tips of the electrodes were made in such a way that a small nerve could be suspended across them, making contact by lying in a meniscus of the calomel saturated Ringer's solution which filled the cells.

Three methods of attack were employed in the study: 1. The nerves were stimulated electrically and their qualitative reflex responses and the thresholds of these responses were determined. The reflex thresholds were compared with the motor fiber threshold of the peroneal nerve low in the leg. It was found that the peroneal

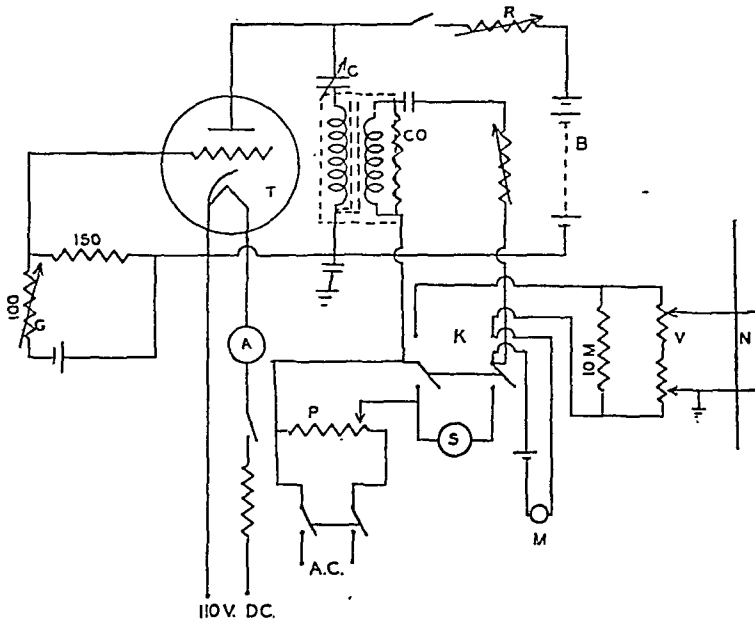


Fig. 1. Thyatron stimulator circuit with mechanisms for calibration of frequency, and for recording the time of closing the stimulating circuit.

V, 412 ohm potential divider mentioned in the text; T, FG 67 thyatron tube; G, 100 ohm variable resistance to vary the grid bias; C, variable condenser which discharges through the tube when the breakdown potential is reached; R, variable resistance used to regulate the frequency of oscillation; CO, shielded coreless Porter inductorium with 10,000 ohm resistance across the secondary terminals; K, double pole double thrown switch which may be thrown in one direction to close the stimulating circuit, or in the other to close the calibrating circuit; S, magnetic loud speaker used for calibration of frequency; P, potential divider in 60 cycle A.C. line used for calibration; M, signal magnet to mark the time of closing the stimulating circuit.

threshold is approximately identical with that of tibialis superficialis as indicated in each case by the contraction of toe muscles. The threshold of the most irritable fibers in either of these nerves is slightly lower than that of the fibers in the triceps nerve which was used first. It was thought desirable to make the comparisons with a motor nerve containing the most irritable fibers from the sciatic. 2. The diameters of the large fibers in the various nerves were measured from histological preparations. 3. The thresholds and conduction rates of the fibers composing the various cutaneous nerves studied were determined with the cathode ray oscillograph.

These were compared with the thresholds and conduction rates of their parent tibial and peroneal nerves. In each case the sciatic was stimulated and the leads to the amplifier taken from the cutaneous branch being studied, or from a distal portion of the parent trunk near the point of departure of the cutaneous branch. After the nerve was placed on the electrodes, it was not disturbed until all determinations on both the branch and parent trunk were completed.

QUALITATIVE REFLEX RESPONSES TO STIMULATION OF THE VARIOUS NERVES. *Ramus cutaneus cruris posterior (posterior nerve)*. Since stimulation of the skin posterior to the gastrocnemius muscle gave as its usual lowest threshold response ipsilateral extension, it was reasoned that stimulation of the nerve supplying this area should evoke a similar response. This nerve, the posterior, is a branch of the tibial. With care about 3 or 4 cm. may be dissected free from the skin. The reflex response was observed in two ways; first, by recording the activity of the triceps and semitendinosus muscles on a smoked paper kymograph; and second, by watching the contraction in the triceps. It was found that simply watching for threshold responses was quite as sensitive as kymographic registration. The smallest threshold contraction usually occurs in the same part of the muscle in all preparations, appearing in the lateral-distal part near the patellar tendon.

The one response to stimulation of the posterior nerve which invariably occurred was that of ipsilateral extension as expressed by contraction of the triceps and gastrocnemius muscles. The triceps always responded more vigorously than did the gastrocnemius. In some animals it was possible also to obtain flexor reflexes upon stimulation of this nerve, but the two types of responses were easily separable in terms of the threshold intensities of the shocks required to evoke them, and the vigor of the responses.

Frequently, ipsilateral extension was the only response obtainable by stimulation of this nerve, and in such cases the semitendinosus usually showed reflex relaxation. In some experiments the intensity of stimulation was carried to more than one hundred times the extensor reflex threshold without changing the character of the response. If flexion appeared at all, it began at about two to two and a half times the threshold for the extensor reflex. In those experiments in which no flexor contraction occurred, the extensor response was as sensitive and appeared to be as strong as in other experiments in which a flexor contraction did occur. The flexor excitation was frequently manifest only as post inhibitory rebound as in figure 4.

Figure 3 shows a record of pure ipsilateral extension accompanied by flexor relaxation. In order to test whether or not the flexor reflex central apparatus was functional a toe of the ipsilateral foot was lightly pinched. The semitendinosus responded with a vigorous contraction. It would seem from these experiments that one of two conditions must prevail;

either the posterior nerve contains no fibers which mediate the flexion reflex, or the center for flexion was so greatly inhibited by the reciprocal innervation of the extensor mechanism that the flexion reflex failed to appear. Not only did the flexor muscle fail to respond by contraction, but it relaxed, indicating that its previous tonic discharge was diminished. The first of the conditions postulated above cannot be true, because in some experiments a flexion reflex is obtained upon stimulation of this nerve, the threshold for this response being high as previously mentioned. Therefore, the other alternative must hold: the flexor muscles did not respond because their center was so greatly inhibited.

Nervus cutaneus dorsi pedis lateralis (dorsal nerve). Stimulation of this nerve which is a branch of the peroneal supplying the skin of the dorsum and dorsolateral part of the foot gives flexion as practically its only reflex response. It is true that brushing the dorsolateral region evokes a mixed response, but with the microscope almost all of the nerve filaments from this area can be traced into the tibial nerve, not into the dorsal. However, in some of the records of reflex contractions evoked by stimulation of this nerve the triceps record shows a barely perceptible rise above the base line while the flexor muscle responds with a vigorous contraction. In other records, there is no trace of a response in triceps. Certainly one can say without hesitation that almost all of the response is in the flexor muscle.

In these experiments, the hip flexor component of triceps was denervated. Inspection of the triceps muscle during an extensor reflex contraction, and during a flexor reflex contraction shows clearly that these two responses in triceps occur in different parts of the muscle, each in its own end. The distal half contracts during the extensor reflex, and the proximal part during flexion. The innervation of triceps is likewise in two parts; part by way of the crural nerve, and part by way of the triceps nerve from the sciatic. The crural nerve apparently is distributed only to the flexor end of the muscle. The triceps, however, is divided into branches, chiefly two, the largest of which courses distally to the extensor end, and the smaller branch courses to the proximal part. By cutting the crural, and the proximal branch of the triceps nerve it is possible to denervate the flexor part of the muscle, perhaps completely, without interfering with the nerve supply to the extensor end.

Figure 5 shows the responses which may be obtained upon stimulation of the dorsal nerve with the flexor part of triceps denervated. In A the rises in the extensor line are slight, but the flexor contractions are quite strong. B is from another experiment. The extensor reflex was shown to be functional by stimulating the skin posterior to the gastrocnemius muscle. A strong contraction in the extensor end of triceps resulted.

It might be argued that the slight activity in the extensor record is in reality flexor, and due to an incomplete denervation of the flexor com-

ponent. The fact remains, however, that the small rise in the extensor line occurred at a lower threshold than did the contraction in semitendinosus. This indicates that it is unlikely to be a flexor response. The extensor response to stimulation of this nerve, however, is extremely small if it occurs, and the primary reflex response to stimulation of the dorsal nerve is flexion.

Ramus cutaneus medialis inferior (medial nerve). This nerve is double, being in reality two branches from the tibialis superficialis. They leave the parent trunk near the same level, slightly below the middle of the gastrocnemius muscle, course distally and supply the skin in the region of and medial to the Achilles tendon. The two branches supply contiguous areas, and are similar in the reflex responses that they evoke. They are similar also in each of their other properties studied. The reflex response to stimulation of these nerves is qualitatively similar to that found in the case of the posterior.

The primary response is, therefore, ipsilateral extension at low threshold and the addition of flexion at higher threshold. One difference is to be noted: though one always obtains extension as the lowest threshold response, the flexion which appears when the intensity of stimulation is sufficiently raised is more vigorous than in the case of the posterior nerve, and appeared in all of the experiments. This would seem to mean that these nerves contain a larger proportion of fibers which mediate the flexor reflex than does the posterior.

Ramus cutaneus plantae lateralis (plantar nerve). This also is a branch of tibialis superficialis. It leaves the parent trunk at the level of the plantar aponeurosis and supplies the skin of the sole of the foot. Though it gives as its lowest threshold response ipsilateral extension, the responses are somewhat different in character from those of other nerves studied. Before dissection of the leg, stimulation of the skin of the sole gives rise to a powerful bilateral extensor thrust. This response was described by Baglioni (1904). After the nerve is dissected free and the muscles prepared for the registration of reflex responses, stimulation of the nerve results in a low threshold extensor contraction as shown in figure 7 but it has lost the nature of a thrust. Flexion appears also at about 1.8 to 2 times the extensor threshold and upon the use of strong shocks it becomes very powerful in contrast to the weak reaction inhibited almost to extinction in the case of the posterior nerve.

The extensor thrust occurs first and more powerfully in the hip extensor muscles, followed by contraction of the knee extensors. Efforts have been made to register it both from the gracilis major and triceps muscles, but in no case has it occurred after dissection and fixation. Records of the thrust were made by inserting a pin into the patellar tendon through a small hole in the skin and recording the extensor movement of the whole thigh.

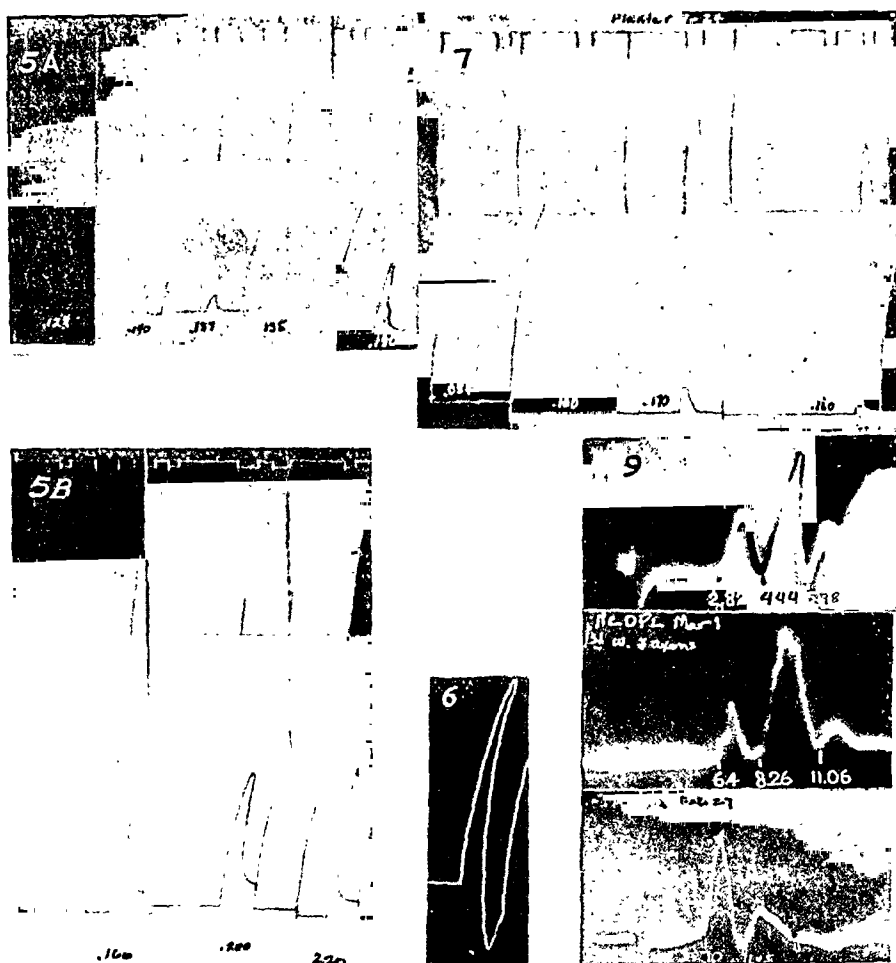


Fig. 5A and B. Reflex responses to stimulation of the dorsal nerve. Records from two experiments. In each record: upper myogram, triceps and lower myogram, semitendinosus. Record A shows a trace of extensor response in addition to the flexor contractions and at a lower threshold. Record B shows flexor contraction only. Numbers as in figure 4.

Fig. 6. Extensor thrust evoked by stimulating the skin of the plantar surface. Record of movement of the leg, attachment being made to the patellar tendon over the knee.

Fig. 7. Reflex responses to stimulation of the plantar nerve. Upper myogram, triceps. Lower myogram, semitendinosus. Numbers as in figure 4.

Fig. 9. Action potential records from the posterior nerve (upper), dorsal nerve (middle) and the peroneal (lower).

The numbers under the records indicate the time in ms. from the delivery of the shock to the beginning of the elevation designated.

The conduction distances were: posterior 75.7 mm.; dorsal 135 mm.; and peroneal 96 mm.

All records were made with linear time axis.

The skin of the plantar surface was stimulated by rapidly repeated shocks. Figure 6 shows the contraction.

Ramus cutaneus cruris lateralis (lateral nerve). This nerve is a branch of the peroneal. It leaves the parent trunk just above the knee and courses downward and laterally supplying the skin covering the lateral part of the crus. When it is stimulated with shocks of suitable intensities, responses are evoked in both the extensor and flexor muscles, the flexor response being the greater when maximal. The extensor response, however, occurs at a lower threshold than does the flexor. Threshold relationships will be set forth more definitely in the section on thresholds.

Reflex thresholds. In order to obtain reflex responses by stimulation of as few afferent fibers as possible, i.e., to cause the reflex threshold to approach the threshold of the most irritable afferent fibers which mediate the reflex, it is essential to use repetitive stimulation. This is a necessary corollary to the findings of Eccles and Sherrington (1930). They reported that shocks of a certain intensity which singly will not evoke a reflex response are adequate to elicit a reflex contraction if sent in in pairs, the two shocks being separated by a suitable interval. They found that for the flexor reflex in the cat the two shocks are most effective when separated by 6 to 8 milliseconds (ms.). This interval would correspond to a frequency of 125 to 167 per second. A single impulse arriving at the center by way of an afferent nerve fiber will not excite the center sufficiently to cause a reflex response. An excitatory condition (central excitatory state) must be built up to neuron threshold before there is any discharge from the motoneurons. This can be done in either of two different ways: 1, by a single shock applied to the nerve if the intensity of the shock is great enough to excite a sufficiently large number of fibers that are concerned with the reflex, or 2, by exciting one or a few fibers at a sufficiently rapid rate. In the latter case, when the optimally effective frequency¹ is used, the reflex threshold approaches or becomes the same as the threshold of the most irritable fiber which serves to elicit the reflex. This optimum is approached gradually as the frequency is increased from single shocks or a low beginning frequency. As the frequency increases, within the range below the optimum, the reflex threshold intensity decreases.

The accompanying graph, figure 8, shows the relation that exists between frequency of stimuli and threshold intensity for the ipsilateral extensor reflex evoked by stimulation of the posterior nerve, and for the flexor reflex evoked by stimulation of the dorsal. The curves show that the optimally effective frequency for the extensor reflex is reached at about 120 per second while that for the flexor reflex is about 60. This agrees

¹ The optimally effective frequency means that frequency beyond which further increases fail to further lower the reflex threshold.

very well with the optimal frequency-tension data of Sassa (1921) for the flexor reflex in the frog.

At the optimally effective frequency the extensor reflex threshold intensity becomes practically identical in value with that of the most irritable motor fiber in the peroneal nerve which was used for comparison, whereas that of the flexor reflex remains about 1.6 to 1.7 times as high.

The excitation threshold of nerve fibers, as shown by stimulating a motor nerve and observing the resulting muscular contractions, does not decrease with increasing frequency of stimulation within the range used,

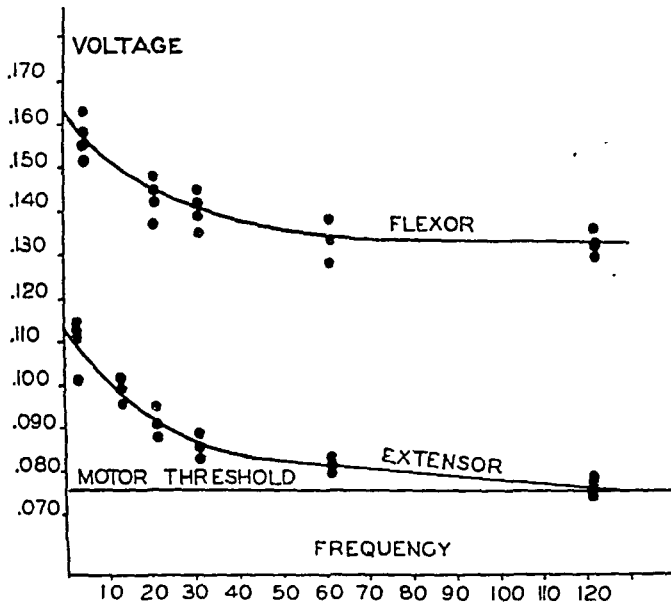


Fig. 8. Chart illustrating the following relationships:

1. That of reflex thresholds to frequency of stimuli.
2. That of the flexor reflex threshold to the extensor reflex threshold.
3. That of the flexor and extensor reflex thresholds to the motor nerve threshold.

Each reflex threshold curve represents the results of one experiment. In the two experiments selected for this chart the motor nerve thresholds were equal.

single shocks to 180 per second. This is the result that one would anticipate on the basis of the data of Erlanger and Blair (1931) which show that the summation interval in large nerve fibers at room temperature is only about 0.2 to 0.6 ms. Very much higher frequencies than any used in this study would have to be employed in order to lower the threshold of the fibers. The depression phase following a subthreshold shock is of much longer duration, being of the order of 3 to 5 ms. The depression phase is similar in this respect to the relatively refractory period following the discharge of an impulse. The real danger in the use of high frequencies, therefore, would be that of sending in shocks during the period of depression

and finding a higher threshold. In view of the data cited, this does not become a factor unless stimuli more frequent than 200 per second are employed. The frequencies used in the experiments here reported usually have not been higher than 120 per second.

The reflex threshold could never approach the motor nerve threshold unless the most irritable afferent fiber which serves to evoke the reflex is as irritable as the lowest threshold motor fiber in the nerve which is used for

TABLE 1
Reflex and motor nerve thresholds

NERVE	EXTENSOR THRESHOLD	FLEXOR THRESHOLD	MOTOR THRESHOLD
Posterior 1-15	0.073 (60 sec.) 0.069 (120 sec.)	No response	0.068
Posterior 1-16	0.078 (60 sec.) 0.075 (120 sec.)	No response	0.075
Posterior 2-10	0.090 (60 sec.) 0.084 (120 sec.)	0.178	0.082
Posterior 2-27	0.082 (60 sec.)	0.147	0.077
Dorsal 2-27	None (60 sec.)	0.140	0.084
Dorsal 1-20	None (60 sec.)	0.128	0.082
Dorsal 1-22	None (60 sec.)	0.124	0.074
Dorsal 3-14	0.128 (60 sec.) (trace)	0.135	0.084
*Medial 1-24	0.042 (60 sec.)	0.080	0.040
Medial 1-26	0.083 (60 sec.)	0.167	0.078
*Plantar 1-24	0.048 (60 sec.)	0.080	0.044
Lateral 3-20	0.100 (60 sec.) 0.096 (120 sec.)	0.110 0.111	0.094
Lateral 3-23	0.085 (60 sec.) 0.080 (120 sec.)	0.129 0.129	0.075 0.075

* Made with silver electrodes. All other determinations made with calomel electrodes.

The thyatron stimulator was used in all of the experiments listed above. The values are expressed in arbitrary units of the applied potential.

comparison. The reflex threshold data and the motor nerve threshold data contained in table 1 are typical of those found for the various nerves that have been studied.

A further significant fact which one may glean from the table is that the threshold of the flexor reflex is higher if it is evoked from a nerve whose main response is extension than if evoked by stimulating a nerve whose main response is flexion. Table 2 will show this more clearly. This difference is, no doubt, significant and is probably to be explained as due to the inhibitory influence exerted by the extensor afferent fibers upon the center for flexion. It is to be recalled in this connection that in the myo-

grams registering the movement of the triceps and semitendinosus muscles when the posterior nerve is stimulated one frequently sees only flexor relaxation accompanying extensor contraction. When the flexor does contract the contraction is usually preceded by a phase of relaxation, the relaxation occurring simultaneously with the extensor contraction.

The truer values for the flexor reflex threshold, therefore, are no doubt those found by stimulation of a nerve such as the dorsal which does not evoke any appreciable extensor contraction. The most irritable fibers which evoke the flexion reflex then are fibers whose thresholds are 1.6 to 1.7 times the threshold of the largest motor fibers.

OBSERVATIONS MADE WITH THE CATHODE RAY OSCILLOGRAPH. *Thresholds and conduction rates.* By the use of the cathode ray oscillograph to study the action potentials of the various nerves the following types of data were obtained: 1. The thresholds of the most irritable fibers and of those con-

TABLE 2
Ratio of flexor reflex threshold to motor threshold

EXTENSOR NERVE		FLEXOR NERVE	
Posterior	2.17	Dorsal	1.66
	1.90		1.56
	2.92		1.68
Medial	2.17		1.70
	2.14		1.63
			1.85
Plantar	2.17		1.69
			1.62
Average..... 2.24		1.67	

tributing to the various elevations in the action potential record both of the cutaneous nerve and the parent trunk have been determined; 2, the conduction rates of the fastest conducting fibers in the nerve and the first fibers contributing to the various elevations have been ascertained; and 3, the configuration of the action potential wave complex has been observed. In tables 3 and 4 certain relationships derived from the findings are summarized.

Configuration of the action potential records. Figure 9 reproduces in reduced size the action potential records of the posterior, dorsal and peroneal nerves. Both in height and area, the first elevation of the posterior nerve action potential is greater than that of the dorsal with respect to the second elevation, or the second elevation of the dorsal is relatively the greater. The ratios of heights and areas of the first two elevations as measured from the original pictures are shown in table 5.

By counting the single axon action potentials as they appeared on the

screen of the oscillograph while the strength of stimulation was gradually being increased, it was seen that only three axons contributed to the first elevation of the dorsal while about 12 to 15 axon potentials could be identified as they joined the first elevation of the posterior. Microscopically, these figures approximately correspond to the number of fibers more than $10\ \mu$ in diameter in each of the nerves.

TABLE 3

Ratio of elevation thresholds to the threshold of the first elevation of the parent nerve

CUTANEOUS CURVE	ELEVATIONS				PARENT NERVE	ELEVATIONS			
	1	2	3	4		1	2	3	4
Post. 1-17	1.00	1.88	3.71	22.66	Tib.	1.00			
Post. 1-10	1.07	1.88	3.13	10.34	Tib.	1.00			
Med. 1 2-8	1.00	1.73	3.42	5.87	Tib. S.	1.00	1.63	2.85	8.00
Med. 2 2-8	0.98	1.59	2.10	8.00	Tib. S.	(same nerve)			
Plant 2-23	1.00	1.77	4.50	10.40	Tib. S.	1.00			
Averages.....	1.01	1.77	3.37	11.45					
Lat. 3-20	1.45	2.30	5.44		Per.	1.00	2.22		
Lat. 3-23	1.07	1.48	2.60	5.20	Per.	1.00	1.56	2.52	
Dors. 3-1	1.30	1.53	2.69	5.80	Per.	1.00	1.67		
Dors. 3-14	1.38	1.60	2.35		Per.	1.00	1.56	2.52	
Dors. averages.....	1.34	1.57	2.52	5.80					
All parent nerve averages taken together.....						1.00	1.73	2.66	8.00

The data on the lateral nerve are separated from the others, and not included in the averages because of the fact that this nerve supplies a transitional area of skin, and exhibits greater variations than do the other nerves.

In all of the nerve action potentials, one frequently sees a few scattered axon potentials which do not form a real elevation. This is especially true beyond the third elevation. It is, therefore, sometimes difficult to decide just what group of potentials to count as an elevation. This is the reason for the large variation in the figures listed for the fourth.

The tables show that the threshold of the most irritable fiber in this particular posterior nerve was 1.07 times that of the most irritable fiber in the parent trunk. This was the highest ratio found for any posterior nerve, the more usual ratio being 1.00. The threshold of the most irritable fiber in the dorsal nerve whose action potential is shown, however, is 1.30 times that of the most irritable fiber in the parent trunk. The conduction rate of the fastest fiber in the first is 34.4 m.p.s. as against 25.3 for the fastest fiber in the latter.

The peroneal record which is added in order to have the action potential of a large mixed nerve for comparison has a relatively much greater first elevation than does either of the cutaneous nerve records. It is to be

TABLE 4

Ratio of conduction rates of elevations to that of the fastest elevation of the parent nerve

CUTANEOUS NERVE		ELEVATIONS			PARENT NERVE	ELEVATIONS		
		1	2	3		1	2	3
Post.	1-17	1.00	1.49	2.83	Tib.	1.00		
Post.	1-10	1.12	1.80	2.40	Tib.	1.00		
Med. 1	2-8	1.02	1.53		Tib. S.	1.00	1.51	2.42
Med. 2	2-8	1.00	1.52		Tib. S.	(same nerve)		
Plant	3-20	1.04	1.65		Tib. S.	1.00		
Averages.....		1.04	1.60	2.62				
Lat.	3-20	1.12	1.70	2.67	Per.	1.00	1.59	
Lat.	3-23	1.00	1.29	2.35	Per.	1.00	1.40	2.15
Dors.	3-1	1.28	1.66	2.21	Per.	1.00	1.59	
Dors.	3-14	1.22	1.71	2.00	Per.	1.00	1.64	
Dors. averages..		1.25	1.69	2.11				
Parent nerve averages.....						1.00	1.55	2.29

TABLE 5

Ratios of the elevation heights (voltages) and areas of the second and first elevations

NERVE	HEIGHT	AREA
Posterior.....	2.00	1.44
Dorsal.....	2.31	6.29
Peroneal.....	0.30	0.41

TABLE 6

	POSTERIOR 3-26	MEDIAL 3-4	PLANTAR 3-4	LATERAL 3-28	DORSAL 3-5
Diameter of largest fiber in microns...	19.0	17.5	18.1	15.0	12.8
Number of fibers above 12 microns.....	17	10	9	7	1

remembered in this connection that the peroneal nerve contains many fibers, both motor and sensory, which supply muscles. This, no doubt, accounts for the enormous first elevation present.

Correlation between reflex and action potential data. When one considers

together the two sets of data, that pertaining to the reflex thresholds, and that relating to the action potential elevation thresholds, it is at once suggested that the fibers which mediate the ipsilateral extensor reflex may be the ones which form the first elevation, while those which evoke the flexor reflex are responsible for the second. However, further experiments show that this is only partially true. The most irritable of the fibers mediating the extensor reflex are among those giving rise to the first part of the first elevation, but the range through which fibers that mediate this reflex extend is broader than that of fibers contributing to both of the action potential elevations. Isometric records of the reflex muscle contraction show that the muscle continues to develop tension through a range of fibers from those similar to the most irritable motor fibers to fibers whose thresholds are about three times as great. This means that the fibers which mediate this reflex extend at least through the first two elevations, and into the third. Stated in another, and perhaps better way, the fibers which mediate this reflex of the ipsilateral extensor are fibers which conduct at rates ranging from about 40 to 14 meters per second.

Similarly, those fibers which mediate the flexor reflex extend through a band of the fiber spectrum which is broader even than the range of fibers which mediate the extensor reflex. The most irritable of the fibers which mediate the flexor response have about the same irritability as those which contribute to the first part of the second action potential elevation, but as one increases the intensity of the stimulation, the tension developed by the muscle continues to show increments until a stimulus strength 8 to 10 times the intensity required to excite the largest motor fibers is reached. This means that the fibers extend deep into, or perhaps through the fourth or B elevation. The latter is consistent with the findings of Heinbecker, Bishop, and O'Leary (1933) that the fibers which mediate the sense of pain lie largely in the B range. This statement must not be taken to mean that the flexion reflex is a nociceptive reflex in its entirety. In fact, evidence is presented in another part of the study showing that in the near threshold range this is distinctly not the case. However, it very probably is true that the nociceptive fibers do contribute to the flexion reflex, though the range of fibers which evoke the flexion reflex is broader than that of fibers which carry pain impulses. To describe the fibers which evoke the flexor reflex in terms of conduction rate, one may say that they are fibers which conduct at rates of 27 to perhaps 6 or less m.p.s.

Morphological correlations. Osmic acid preparations of samples of all the different nerves studied were examined, and the diameters of the large fibers were measured. All of the myelinated fibers of one section of the posterior nerve were measured, but as only the fibers of the low threshold range can clearly be correlated with the other findings in this study, only the diameters of the large fibers will be given. Table 6 gives typical data

on the largest fibers from sections of selected examples of the various nerves.

All of the nerves show considerable morphological variations. The largest fiber, for example, in the posterior is sometimes as small as $17.5\ \mu$, but has been found to be as large as $24\ \mu$. However, in no specimen of the dorsal has a fiber larger than $13.8\ \mu$ been found and the largest fiber may be as small as $10.8\ \mu$. Out of five sections of this nerve measured only one contained as many as two fibers as large as $12\ \mu$, and two contained no fibers that large. In the sciatic, peroneal, and tibial nerves studied, the largest fibers lay within the same diameter range as that reported for the posterior, i.e., 17.5 to $24\ \mu$. Therefore, the morphological findings appear to confirm completely the reflex and oscillographic data reported. The nerves which contain fibers of low threshold and rapid conduction, and which evoke the low threshold extensor response contain fibers within the size range of the largest fibers in the sciatic. The nerve which does not evoke the extensor reflex, or only to an almost imperceptible degree, but whose main reflex response is flexion, and which exhibits a higher threshold and slower conduction rate, does not contain such large fibers.

Central time relations. The finding that the optimally effective frequency of stimulation for the extensor reflex is in the region of 120 per second, while that for the flexor reflex is about 60 seemed to indicate that there is a fundamental difference in the time relations of the central processes for the two reflexes. In order to ascertain whether or not this is the case the central reflex time for each of the two reflexes was measured by recording them simultaneously upon a fast moving drum. The reduced reflex time for the ipsilateral extensor reflex was found to be 21.9 ms., while that for the flexor reflex is 34.4. In these measurements only those are considered for which stimuli of intensities of 1.36 times the flexor reflex threshold and greater were employed. It was found that when weaker stimuli were used there was great fluctuation in the latent period, especially that of the flexion reflex. One measurement of a record made by stimulation with shocks 1.05 times the flexion reflex threshold showed a gross flexion reflex latency of 260 ms. However with shocks of 1.36 times flexor reflex threshold and stronger, the reduced reflex time was fairly constant, with the flexion time still showing the greater variation.

At any rate, one unchanging result appeared in the records. Regardless of the strength of stimulation used and all other conditions, in every record of simultaneously recorded flexor and extensor reflex responses the extensor latent period was shorter than that of the flexor by at least 12 ms., and usually more. These figures on reduced reflex time are slightly longer than those of Buchanan (1908). She recorded the ipsilateral extensor (gastrocnemius) reflex of *Rana temporaria*, and reported a reduced reflex time for the electrical response usually between 12 and 20 ms. This difference of 3 or 4 ms. may possibly be a species difference.

The two kinds of data on the central time relations of the two reflexes, namely, 1, that the optimally effective frequency for the extensor reflex is higher, and 2, that the latent period is shorter, indicate that central excitatory state builds up more quickly in the extensor center, but is of shorter duration or dissipates more rapidly. The extensor center exhibits the property of inertia, therefore, to a less marked degree than does the flexor center.

DISCUSSION. The differences in speed of reaction of the flexor and extensor reflex arcs, peripherally and centrally, may conceivably be an instance of the difference between postural and locomotor mechanisms. It seems significant that Brondgeest (1860) found that in the frog, corresponding with the habitual posture of squatting, postural tonus is chiefly in the flexor muscles. J. Hay (1901) has reported that in the rabbit the motor fibers to red muscle average about 5μ smaller than the motor fibers to pale muscle. It is known that the red muscles are slower in their contraction than are the pale muscles, and there is evidence which indicates that they are postural in function (D. Denny Brown, 1929). It appears a worth while speculation, therefore, that the ipsilateral extensor reflex in the bullfrog which is evoked, par excellence, by stimulation of the posterior nerve may be a locomotor reaction, while the non-nociceptive part of the flexor response may be postural.

Another phase of the problem which this study illuminated is the relative difficulty which other workers have experienced in evoking the ipsilateral extension reflex, and the variations in the quality of response they have obtained upon stimulation of large mixed nerves. Ranson (1931) and Ranson and Hinsey (1931) have doubtless inferred the real reason for the heterogeneity of responses. They attributed the mixed responses and difficulties of interpretation to the functional mixture of afferents excited.

It has been pointed out by Sherrington (1894) that the muscle afferent fibers supplying both the flexor and extensor muscles of the cat are large fibers, and there is no regularity of difference between them. If this is true in the frog, there are at least three functional groups of afferent fibers in the leg of the bullfrog which lie in the same threshold range. They are: first, proprioceptive fibers from the extensor muscles; second, similar fibers from the flexors; and third, large cutaneous fibers which evoke the extensor reflex. It is not surprising then that even in the low threshold range inconstant reflex responses are obtained when a large mixed nerve is stimulated. In the posterior and the other *cutaneous nerves containing large fibers*, however, there is apparently a quite broad band of fibers which are functionally homogeneous, thus accounting for the constancy of the response to their afferent stimulation.

All of the results of these experiments are consistent with the doctrine of "specific nervous energy." Excitation of a certain afferent fiber evokes only one type of muscular reflex response as shown by the muscles of the

hind limb. In one experiment, the frequency of stimulation of the posterior nerve was increased to more than 500 per second. The intensity was such that only low threshold fibers were excited. The triceps muscle responded by contraction. The semitendinosus remained quiet. All of the results in this study seem to indicate that the kind of reflex response evoked, and by inference the sensation experienced, when a nerve is stimulated depends upon the central distribution of arriving impulses, and not upon the frequency of arrival or other factors.

SUMMARY

The cutaneous afferent fibers which evoke ipsilateral extensor and flexor reflexes in the hind limb of the bullfrog have been identified and certain correlations derived.

The stimulation of different areas of the skin of the hind limb of the bullfrog evokes qualitatively different reflex responses in the muscles of that limb. Excitation of the fibers of the nerves supplying these different skin areas elicits corresponding reflex responses.

The optimally effective frequency of stimuli for the ipsilateral extensor reflex is found to be about 120 per second; that for the flexor reflex is about 60. The threshold of the extensor reflex at a frequency of 120 per second is similar to that of the most irritable motor fibers in the sciatic nerve. The threshold of the flexor reflex at its optimally effective rate is about 1.6 to 1.7 times this value.

The cathode ray oscillograph discloses that the cutaneous nerves which primarily evoke the ipsilateral extension reflex contain fibers which are as irritable as the lowest threshold fibers in the sciatic nerve, and whose rates of conduction are similar to those of the most rapidly conducting fibers of the sciatic. The nerve which does not evoke the extensor reflex, or to only a very small degree, but whose main response is flexion does not contain fibers with such low thresholds and rapid rates of conduction.

Morphologically, the nerves which best evoke the extensor reflex are found to contain fibers as large as the largest fibers in the sciatic, while the largest fibers in the nerve which evokes the flexor reflex as practically its only muscle response average 6 to 10 μ smaller in diameter.

The reduced reflex time for the extensor reflex is found to be shorter than for the flexor reflex. This finding considered together with the higher optimally effective frequency of stimulation and the fact that the afferent fibers are larger indicate that both the central and afferent peripheral processes are faster for the extensor reflex than for the flexor.

These differences may signify that they are postural and locomotor mechanisms.

The flexion reflex elicited by excitation of cutaneous nerve fibers, in the light of certain findings in this study, cannot be considered as entirely nociceptive in origin.

In the fiber "spectrum" the band occupied by the extensor reflex afferent fibers ranges from fibers which conduct at rates from about 40 to 14 m.p.s.; that occupied by the flexor fibers from about 27 to 6. The ranges, therefore, are wide and overlap. When excited by distinctive strengths of stimuli an increase in rate is without effect on the quality of the responses. This may be considered as evidence supporting the doctrine of "specific nerve energy."

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THE EFFECT OF TOTAL THYROIDECTOMY UPON EXPERIMENTAL DIABETES INSIPIDUS IN DOGS

WILLIAM MAHONEY AND DONAL SHEEHAN¹

From the Laboratory of Physiology, Yale University School of Medicine

Received for publication February 7, 1935

While investigating the experimental production of diabetes insipidus, the problem of correcting the upset water balance presented itself for study. Reasons for believing that the thyroid is implicated are to be found in recent literature and the present communication gives a brief account of experiments bearing upon this phase of the problem.

In 1910 Crowe, Cushing and Homans (1) showed in dogs that transection of the pituitary stalk resulted in a transient severe polyuria, and shortly thereafter Cushing and Goetsch (2) reproduced the phenomenon by placing a "silver clip" upon the pituitary stalk. Although polyuria has been noted after hypophysectomy, and following lesions of the hypothalamus, more consistent and lasting effects have been produced by damage to the stalk. We have depended upon this last method for the present study. The duration of the polyuria has been variable, so for this investigation only animals which showed polyuria for at least two weeks were selected for subsequent thyroidectomy.

The importance of endocrine interrelationships has been recognized for some time and it has been felt more recently, as facts have been unfolded, that the controlling mechanism is to be sought in the pituitary body. One of the first connections noted was that between the pituitary and thyroid. In 1889 Rogowitsch (3) described enlargement of the pituitary body following thyroidectomy. This was confirmed by Herring (4) in 1908, who noted in particular an "increased activity" of the pars intermedia with a great increase in the number of hyaline bodies in the pars nervosa. Crowe, Cushing and Homans (1) in 1910 observed the reversal, viz., a hyperplasia of the thyroid in the first 48 hours following hypophysectomy, but later functional involution of the thyroid with excessive accumulation of colloid in the vesicles.

In 1920 Strauss (5) reported the case of a boy who developed diabetes insipidus at the age of nine years. When the patient was thirteen years old the polyuria and excessive thirst progressively disappeared with the gradual onset of myxedema. At fifteen years of age the boy was treated by thyroid gland administration with amelioration of the myxedematous

¹ Rockefeller Fellow.

state, but no mention was made concerning the subsequent water balance. The patient died of pneumonia in his twentieth year. No post-mortem examination was made. Barnes, Regan and Bueno (6) in 1933 noted that the marked diuresis which occurred in normal animals following the injection of extracts from anterior bovine pituitaries, was not observed in thyroidectomized dogs. They suggested that the diuresis was the result of thyroid activity. Their observation has been confirmed by Biasotti (7).

METHOD. Healthy mongrel mature and immature dogs were the subjects for study. They lived in the laboratory in large metabolism cages for two to four weeks before any operative procedure was undertaken. Daily measurements were made of the total fluid intake and urinary output. No direct estimations of fluids lost by way of intestines, skin and lungs were made, but the "insensible" loss was calculated by computing the difference between the fluid intake and urinary output. Diets were kept constant throughout the experiment.

The operative procedures were carried out under sodium amytal anesthesia. A median scalp incision was made; the right zygoma was resected; the right temporal muscle was incised and reflected downward as a flap, later to be used in the closure; the bone over the right parietal and temporal regions was removed, and the dura opened widely. The right temporal lobe was elevated and the pituitary body exposed, so that a silver clip was applied to and closed about the pituitary stalk under direct vision. There was usually no bleeding, and closure was done by resuturing the divided temporal muscle and scalp. Within twelve hours the animals recovered sufficiently from amytal anesthesia to begin drinking. During the preceding period of unconsciousness they passed considerable quantities of urine.

Thyroidectomies were performed through a midline skin incision and care was taken to leave the parathyroid glands intact. The excised tissue was in all instances verified histologically. On no occasion was there any manifestation of tetany. The dogs were surprisingly active after thyroidectomy, and showed no gross signs of thyroid privation. There was, however, a remarkable and steady loss of weight after removal of the thyroid. It was found that, after prolonged thyroid feeding, diarrhea frequently followed. In the observations presented thyroid feeding was discontinued immediately upon the appearance of diarrhea.

OBSERVATIONS. Following clipping of the pituitary stalk in dogs, an immediate elevation of the fluid intake and urinary output invariably occurred. The daily measurements reached about ten times the pre-operative readings, but frequently fell after forty-eight hours to five or six times the original quantities. The condition of polydipsia and polyuria then persisted at this level for several weeks and, in some cases, for a period of two months.

In some experiments the postoperative fluid intake was limited to the

average quantity ingested daily before operation. Under these conditions polyuria was not manifest, although the animal showed all signs of excessive thirst. The polyuria appeared immediately the limitation of fluid intake was removed.

The following protocol illustrates the striking effect of thyroidectomy and subsequent thyroid feeding in an adult dog in which the water balance had been disturbed previously by occlusion of the pituitary stalk. Figure 1 shows graphically the details of this experiment.

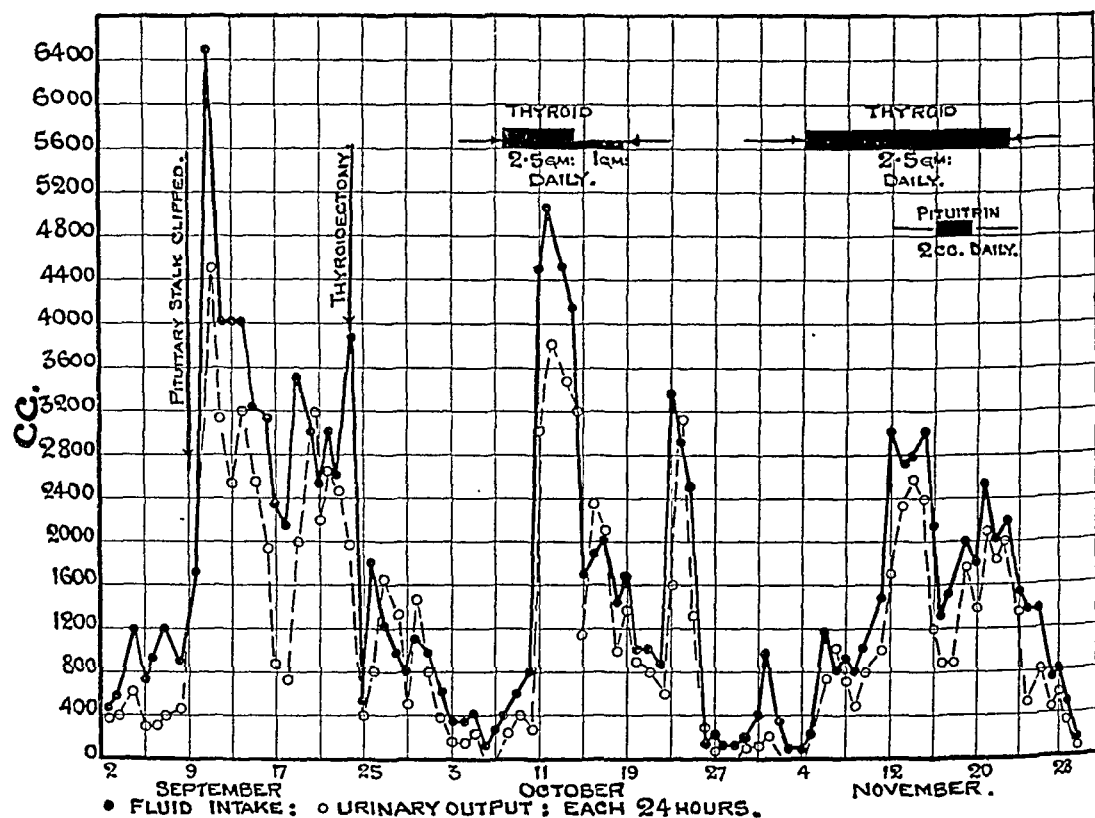


Fig. 1. To show the effect of thyroidectomy and subsequent thyroid feeding on the upset water balance of an adult dog, weight 14.9 kgm., in which the pituitary stalk had been previously occluded.

An adult mongrel bitch weighing 14.9 kgm. before operation had a daily fluid exchange averaging 800 cc. intake and 400 cc. output. Immediately following occlusion of the pituitary stalk the fluid intake rose to 6500 cc. and the output to 4500 cc. per day. After three days the fluid exchange dropped somewhat but remained at a high level, fluctuating between 2500 and 3800 cc. per day. Fifteen days after occlusion of the pituitary stalk total thyroidectomy was performed. Within twenty-four hours the intake had dropped from 3800 cc. to 500 cc. and the output from 2000 cc. to 600 cc. There then followed a gradual fall of the fluid exchange almost to the base line so that the animal was ingesting not more than 200 cc. of fluid daily and the urinary output was so scant as scarcely to be measurable. Replacement therapy

with desiccated whole thyroid gland, 2.5 grams daily by mouth, was begun. Within three days the daily fluid intake remounted from 400 cc. to 5000 cc. and the output from the base line to 3800 cc. It maintained this high level until the thyroid administration was lowered to 1 gram daily, following which the intake dropped to 1700 cc. and the output to 1200 cc. After eleven days of thyroid therapy all glandular administration was stopped, and apart from a spontaneous and unexplainable three day elevation in fluid exchange which occurred exactly simultaneously in other animals in the same laboratory, there was a gradual and steady fall of the fluid exchange to the base line. This low level was maintained for nine days when thyroid gland administration (2.5 gm. per day) was begun again. The rise of fluid exchange at this point, instead of being abrupt was more gradual and the fluid intake ultimately reached a height of 3000 cc. and the output 2600 cc. The thyroid therapy was continued, and, in addition, pituitrin (obstetrical) 1 cc. was given subcutaneously twice daily over a period of three days; during this time the intake dropped to 1300 cc. and the output to 900 cc. After withdrawal of the pituitrin, while the thyroid administration continued, the fluid exchange remounted to 2500 cc. All thyroid feeding was discontinued and the fluid exchange once again fell to a base line level. The animal is still alive and has lost 5 kgm. in weight ($\frac{1}{3}$ of its initial body weight) since thyroidectomy.

The sharp fall in the fluid intake and output following thyroidectomy and the subsequent reappearance of polyuria and polydipsia during thyroid gland administration, illustrated in the above protocol, has occurred without exception in five animals in which the above procedures were carried out after occlusion of the pituitary stalk.

When thyroidectomy was performed simultaneously with occlusion of the pituitary stalk there followed moderate polyuria and polydipsia for a brief period of twenty-four to forty-eight hours, and these states were effectively reproduced by subsequent thyroid administration.

It became obvious that studies of the water balance were required after thyroidectomy alone without previous occlusion of the pituitary stalk, as well as of the effect of thyroid gland administration to normal dogs. Figure 2 illustrates that thyroidectomy is followed by no demonstrable effect upon the urinary output of the normal dog. The intake was exaggerated more than the output after thyroidectomy. The insensible loss of this animal must therefore have been considerable since this subject (a puppy aged 5 months) gained no weight over a period of three months. The difference in the response of water metabolism to thyroid feeding before and after occlusion of the pituitary stalk is strikingly demonstrated in this experiment. Figure 3 is presented as an example of several studies upon dogs *without operation* to show the influence of thyroid feeding on fluid exchange. It will be seen that the daily fluid intake and urinary output rose gradually to almost twice the original levels. The dosage of thyroid therapy given was 2.5 grams daily, admittedly a considerable quantity; but this was done in order that the animals might serve as fair controls for the experiments which involved thyroid administration after occlusion of the pituitary stalk.

In addition to polydipsia and polyuria the application of a silver clip to

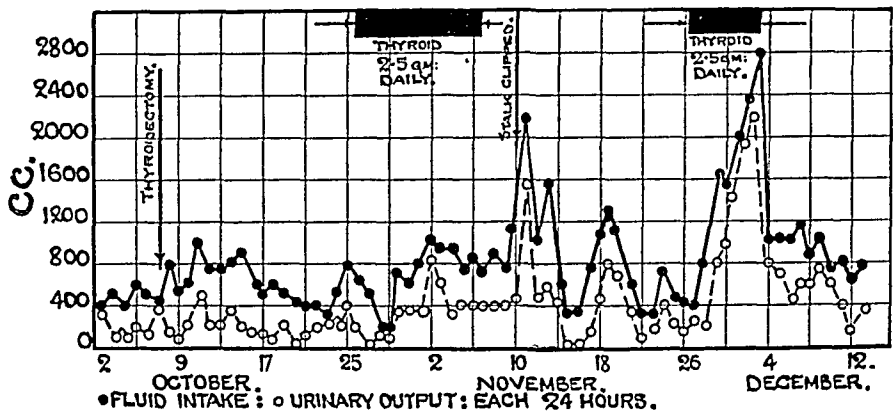


Fig. 2. To show lack of any demonstrable effect of total thyroidectomy upon the water balance of the normal dog. Subsequent occlusion of the pituitary stalk produced a polyuria and polydipsia for one day only. This condition of upset water balance was reproduced by thyroid feeding.

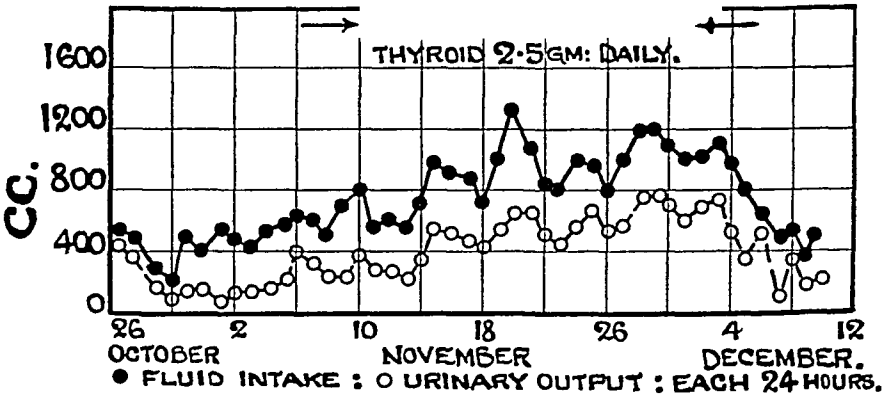


Fig. 3. To show the slight diuresis produced in the normal dog following thyroid administration.

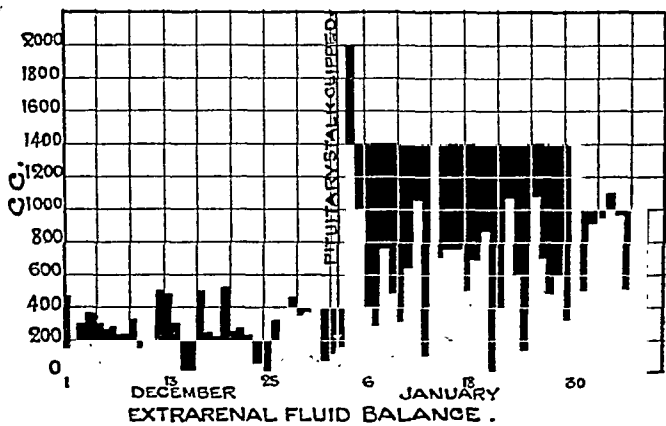


Fig. 4. To show the effect of occlusion of the pituitary stalk upon extrarenal water balance of the dog. The black columns indicate the daily difference between fluid intake and urinary output.

the pituitary stalk was frequently followed by a marked upset in the fluids lost by other routes (intestines, skin and lungs). This took the form of an exaggeration of the excess of fluid intake over urinary output with wide variations in both readings from day to day. Thyroid administration appeared to aggravate this condition. The results indicate a severe imbalance in water metabolism apart from renal excretion. The effect of thyroid on the fluid exchange in tissues might be explained by a rapid breakdown of metabolites with the consequent demand for fluid, but a consideration of the omnipresent dilute urine (specific gravity 1.002 to 1.005) during the periods of polyuria indicates that more fluid had been supplied than should have been necessary to maintain osmotic equilibrium.

SUMMARY

1. In dogs, occlusion of the pituitary stalk with a silver clip is followed by extreme polyuria and polydipsia.
2. This effect is abolished by subsequent total thyroidectomy, and reestablished by oral administration of desiccated whole thyroid gland. Alternate states of extreme polyuria and oliguria could be produced at will.
3. Total thyroidectomy has no demonstrable effect upon the daily fluid intake and output of the normal dog.
4. In dogs with intact pituitary stalk, thyroid feeding has a mild diuretic action. This effect is, however, in no way comparable to the extreme polyuria and polydipsia produced by administration of thyroid gland after occlusion of the pituitary stalk.
5. A severe imbalance of the water metabolism of the tissues apart from the renal mechanism is evident after occlusion of the pituitary stalk in dogs.

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THE EFFECTS OF MAGNESIUM DEFICIENCY ON THE TEETH AND THEIR SUPPORTING STRUCTURES IN RATS¹

HENRY KLEIN, ELSA R. ORENT AND E. V. MCCOLLUM

From the Department of Biochemistry, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland

Received for publication February 27, 1935

When young rats and dogs are fed a ration containing only 1.8 parts per million of the element magnesium, but adequate amounts of other known dietary essentials, they develop a series of symptoms which indicate that in these species, magnesium is essential for life (1), (2), (3), (4), (5), (6), (7).

Such a dietary regimen produces marked pathological lesions in the mouths. The results on rats are herein described.

EXPERIMENTAL. Mouth examinations were made on twenty-four rats which when weaned were placed on the low magnesium diet (2). They were killed or died at various ages as indicated in table 1. The heads were removed and prepared for histological examination using Zenker fixation, eosin staining and hematoxylin. Paraffine imbedding was used for young animals and celloidin for older individuals.

RESULTS. In the control rats, the appearance of the mouth is that shown in figure I. The mouths of rats fed the low magnesium diet present marked differences (fig. II). These appear gradually, as noted in the table, become marked after approximately one month on the diet, and extreme after three months.

Inspection of the maxilla (fig. II), reveals the extent of the change in the tissues. The mucous membranes appear blanched. The gingival tissue, which usually is closely adherent to the bone and the molar and incisor teeth at their gingival margins, is, in the rats fed the low magnesium diet, in both upper and lower jaws, a bulbous mass of smooth whitish grey tissue. Particularly in the lower jaw, the incisor teeth do not lie in close proximity to each other as is usually the case in normal animals but are widely separated at their proximal surfaces by the tissue mass. In the molar regions, darkly colored, brown and yellow, irregularly shaped hard ma-

¹ Aided by grants from the Research Commission of the American Dental Association, and by contributions from members of the Dental Staff of the Johns Hopkins Hospital (Drs. B. L. Brun, H. E. Kelsey, H. H. Streett, W. H. Baish, L. D. Coriell and C. V. Matthews).

terials are imbedded in the tissue mass and protrude above it. The position of these particles of material is that usually occupied by the molar teeth. No structure which suggests the usual molar tooth morphology, is apparent on inspection.

When a small dental probe is applied to the irregularly shaped hard structures imbedded in the tissue masses, these particles become dislodged. When removal of these particles is accomplished, the molar teeth are seen lying deep within the tissue masses. The removed structures appear to be deposits of calculi. The molar teeth after such exposure

TABLE 1

RAT	DAYS ON DIET	GINGIVAL TISSUE SWELLING	MACROSCOPIC DENTAL DECAY
1097	3	None	None
1099	6	None	None
1101	11	None	None
1102	12	Slight	None
A28	12	None	None
A27	12	Very slight	None
A29	14	None	None
A30	14	None	None
A31	15	Very slight	None
A32	17	Very slight	None
A33	17	Slight	None
1106	17	Slight	None
A34	19	Slight	None
A35	22	Slight	None
A36	24	Slight	None
A37	24	Slight	None
A42	36	Marked	None
A43	38	Marked	None
A44	39	Marked	None
A26	52	Marked	None
A25	76	Marked	None
A41	83	Extreme	None
A38	99	Very marked	None
A40	106	Extreme	None

appear relatively unabraded and free from evidences of macroscopic decay. They lie loosely in the tissue mass and may be readily lifted out with forceps.

The incisor teeth appear somewhat longer than in the controls and their labial surfaces are pitted and rough, especially at the gum margin.

Microscopic examination: Controls. The teeth of rats fed the control diet (low magnesium diet plus added magnesium) show on histological section the usual arrangement and structures. As shown in figure III, the three molar teeth lie close together in the antero-posterior position. An-



Fig. I. Maxilla of rat on control diet; age 127 days. *I*, incisors; 1, 1st molar; 2, 2nd molar; 3, 3rd molar.

Fig. II. Maxilla of rat on low magnesium diet; age 127 days.

Fig. III. Arrangement of molar teeth of rat fed control diet. Control for rat A 38 magnification $\times 15$. 1, 1st molar; 2, 2nd molar; 3, 3rd molar.

Fig. IV. Arrangement of molar teeth of rat fed low magnesium diet. Rat A 38 magnification $\times 10$.

Fig. V. Section bone and paradontium control rat 17 days on control diet after weaning. Magnification $\times 120$. *D*, dentine; *P*, paradontium; *B*, bone.

Fig. VI. Section bone and paradontium low magnesium rat on diet 17 days after weaning. Magnification $\times 120$. *D*, dentine; *P*, paradontium; *B*, bone.

teriorly the highest tip of the gingiva lies well below the highest point on the anterior cusp of the first molar. The junction of the tooth epithelium and the gingival epithelium is clearly discernible. The epithelium covering the gingival crest at this point is relatively thin. Beneath this epithelium lies well organized connective tissue. The nuclei are abundant and narrow. These same characteristics are apparent in the gingival crests which lie between the molars. A relatively thin paradontium covers the roots of all the molars. The nuclei of the connective tissue cells of the paradontium are long, narrow and abundant. On the mesial side of the first molar, alveolar bone extends up close to the base of the gingival crest, completely borders the roots against the narrow paradontium, fills the space between the molar roots relatively completely and extends well up to the bases of the gingival crests between all the molars. The bone stains deeply with eosin and only in few areas are there evidences of osteoclastic activity.

The dentine of the molars is uniform in structure, taking a dense hematoxylin stain. The pulp presents the usual characteristics. The molar cusps are worn. No dental decay is apparent. Those portions of the incisors which lie within the jaw (root) present the usual appearance: dentine stained uniformly, the ameloblastic cells and layer unbroken, the subameloblastic layers usual in appearance and below these a layer of dense bone.

Low magnesium rats. With the histological technique used no apparent differences in the paradontium, bone or tooth structures are noted in rats fed the magnesium deficient diet for 3 or 6 days. By the 11th day, however, changes become evident although they are exceedingly fine. Study of the sections indicates that the small observed changes are definite. The nuclei of the cells of the paradontium are more irregular in size and more stellate in shape. The bone bordering the paradontium shows an excessive blue-staining amorphous border over and above in amount that appearing in the controls. No changes are apparent in the developing portions of the incisors at this stage.

After feeding the diet 17 days, the changes, although still of fine character, are more definite and of the same character as described for the earlier stage. The most predominant feature is a change in the character of the cells and intercellular substance of the paradontium. The cells appear larger and are separated from each other by large amounts of pink staining intercellular material. In the controls the cells are long and narrow and closely arranged. These differences may be noted in figures V and VI.

Examination of sections from rats fed the low magnesium diet for three months shows marked differences in the character of the supporting structures of the teeth as well as changes in the teeth themselves. The molar

teeth (fig. IV) lie imbedded in a mass of tissue which stains readily with eosin. Within this mass of tissue lie many spindle shaped cells having large deep blue-staining round or oval nuclei. The usual character of these portions of the gingiva which lie between the molars is changed. The height of the gingival crests lies above the highest point on the molar teeth. The subepithelial connective tissue is increased in amount and is made up of many spindle shaped faintly blue-staining cells having large round or oval nuclei and imbedded in more than usual amounts of faint pink-staining ground substances. The epithelium covering this tissue mass is thickened and shows many extensions into the underlying tissue. Alveolar crests of bone are absent from the usual position about the roots of the molars. The paradontium is not recognized as such for it is intimately fused and appears identical in structure with the bulbous tissue mass. The bone present contains an abundance of marrow spaces and large amounts of an amorphous material taking a deep blue stain. This material chiefly borders the edges of the bone.

The cusps of the molars appear relatively unabraded and are covered in part by heavy deposits of a deep blue-staining substance having some of the characteristics of dental calculus. The dentine of the molars is dense but contains many striations suggesting an intermittent disturbance in its calcification. The pulp tissue is dense and contains very large numbers of closely packed cells, some stellate in shape. The portions of the incisor teeth which lie within the jaw show changes from the controls. The pulp contains large amounts of a deep blue-staining substance, dense and amorphous in structure and continuous with a layer of well organized dentine. Striations in the dentine similar to those in the molars are present. The ameloblastic layer also shows changes. The position of this layer is occupied by a thin deeply blue-staining ribbon-like structure. The layers immediately adjacent labially, usually occupied by the cells of the papillary layer and the stratum intermedium, are filled with a dense deep pink-staining tissue containing many spindle shaped cells with large round or oval nuclei. The connective tissue which lines the dentine on the lingual side of the incisor root, is filled with a thick mass of tissue the characteristics of which are the same as those already described for the tissue surrounding the molar teeth.

DISCUSSION. The question of the necessity and identity of various and specific food substances for physical well-being has occupied the attention of investigators for some time. That many food substances are necessary for the development of normal teeth is, in addition, well recognized. In this connection may be mentioned the significance of adequate and proper levels of calcium and phosphorus, vitamins A, C, D and possibly the B complex. The importance of other inorganic and organic substances when restricted or fed in excess in the diet, in relation to structural excellency of teeth, is as yet to be investigated.

In an attempt to further develop this special field, the effects of strontium inclusion in the diet have already been noted (8). To these data may be added the effects herein described, indicating that the element, magnesium, when restricted in the diet, produces massive pathological changes in the teeth and their supporting structures.

The findings indicate that magnesium restriction in the diet produces its most marked effects upon the character and growth of the cells and intercellular substance of the paradontium. This massive effect upon the membrane which lines the roots of the teeth and adjacent alveolar bone, appears specific with this particular food deficiency. In studies on more than 1500 heads of rats fed a variety of defective diets, none has shown a condition of like character.

The mechanism by which magnesium deficiency induces the described changes in the paradontium is not entirely clear. The studies indicate, however, that changes in the relation of the number of cells to the concentration of intercellular substance appear early and probably first. Undoubtedly the paradontal changes predominate after longer feedings of the deficient diet. These findings, when considered with the chemical studies on this deficiency (5) (6) (7), suggest a possible explanation for the origin of the described pathological changes. The presence of deep blue-staining amorphous material lining the borders of the alveolar bone, especially in the young experimental animals, may be interpreted as a result of precipitation of calcium salts. Although such blue material is present in the sections of control animals, the extent and amounts are less in the latter. It is possible, of course, that the differences in amounts of this substance may be accounted for in part by differences in degree of decalcification in preparation for sectioning. The published chemical findings, however, indicate that favorable conditions are present for precipitation of calcium salts, for there is an increase in the absolute amounts of calcium and, to a lesser extent, of phosphorus in the long bones of the animals.

Coincident with these chemical changes which result in increased weight of the bones, the paradontal changes proceed (note table 1), so that a definite although slight swelling of the tissues about the teeth is present after 15 days. The nature of these chemical findings (calcium and phosphorus retention) makes it improbable that the increase in amount of abnormal paradontal tissue is primarily the result of bone resorption followed by a compensatory increase in the paradontal tissues in an attempt to hold the teeth in place in the mouth. It would appear, therefore, that the effects on the paradontal tissues arise largely from other factors. The picture presented by the sections of the older rats (absence of bone around the teeth and the presence of excessive amounts of abnormal connective tissue) probably results because the tissue proliferation proceeds so rapidly and is of such magnitude as to present the possibility that the teeth are

moved out and away from the alveolar bone by the massive increase in the paradontal tissues.

The chemical findings, although indicating the retention of calcium and phosphorus during early growth, show that longer feeding of the magnesium deficient diet results in excessive loss of calcium and phosphorus through the urine and feces. The character of the alveolar bone in older animals seems to reflect these changes (tendency to highly cancellous character) and may at this later stage in the development of the magnesium deficiency account for part of the excessive paradontal proliferation as an attempt by connective tissue to compensate for decreased density of bone.

The majority of the animals restricted to the deficient diet die early in life. A few survived the spectacular seizures already described (2). These are the animals which show the interesting striations in the dentine which, perhaps, reflect the intermittent character of the seizures and the following recoveries.

SUMMARY

The feeding of a diet low in magnesium is associated with decreased cell content and increased amounts of pink-staining intercellular substance in the paradontium, and also with the formation of a deep blue-staining amorphous material in the bone lining the paradontium after 17 days of feeding.

Sections of jaws of animals fed for three months show absence of bone around the molar teeth and positional substitution by large deep pink-staining masses of tissue containing many spindle shaped cells.

The incisor teeth are surrounded by a similar mass and the tooth structures themselves show marked change, especially the persistently growing incisor roots.

These changes indicate that magnesium is essential for proper formation of the teeth and their supporting structures in the rat.

The possible mechanisms by which these changes arise are discussed.

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GROWTH AND GLYCOGEN CONTENT OF THE FETAL LIVER AND PLACENTA

E. L. COREY

From the Physiological Laboratory of the University of Virginia Medical School¹

Received for publication February 28, 1935

The work of earlier investigators has led to a general understanding of carbohydrate metabolism in the developing embryo and fetus, particularly in regard to the placenta and fetal liver (Needham, 1931). Authors appear to agree on the early glycogenic function of the placenta, first brought to the attention of physiologists by Claude Bernard in 1859. It is indicated furthermore that the initiation of glycogenic activity in the fetal liver occurs when the glycogen concentration in that organ first exceeds that of the placenta.

Demant (1887) was able to detect the presence of glycogen in the livers of term puppies in a concentration as high as 11 per cent. Positive tests were obtained for this substance from the embryos of the cow, sheep and pig by Pflüger (1903). Mendel and Leavenworth (1907) found no trace of glycogen in pig fetuses of 85 to 230 mm. in length, although it was present during the later stages of gestation.

In a study on the fetal rabbit, Lochhead and Cramer (1907) observed that the placental glycogen remained fairly constant until the 24th day of gestation, after which it fell steadily until term. The fetal liver glycogen, on the other hand, increased up to the 25th day of development to a value above that found in the placenta. These investigators concluded that "this . . . represents the date at which the liver assumes its adult glycogenic function." Snyder and Hoskins (1928) also used the rabbit and reported that the glycogen content of whole fetuses rose during the latter third of gestation from a trace to a percentage higher than that in the maternal liver. Little appears to have been added to our knowledge of the subject, therefore, within almost thirty years past.

METHODS. Pregnant rats of Wistar Institute² strain, maintained on a standard diet and fasted for 12 hours, were stunned sufficiently to stop muscular movements but without arresting respiration or cardiac activity.

¹ Acknowledgment is made of aid received in the above investigation from the Committee for Research in Problems of Sex of the National Research Council.

² The rats used in this investigation were of pure Wistar Institute strain, the original stock being obtained through the kindness of Dr. M. J. Greenman.

Immediate laparotomy and rapid excision of maternal and fetal tissue samples were then carried out. Determinations were made of *a*, solid, and *b*, glycogen content of fetal livers by pooling tissues from several fetuses of one litter in each instance; commonly 4 fetuses were used for *a* and 3 for *b* analyses. All fetuses were alive and apparently in good condition at the time of isolation of the liver tissues.

Whole placentae were used for wet weight and total solid data as well as for glycogen determinations, since it was not feasible to separate fetal and maternal portions of that organ. Analyses for glycogen were made according to the modification of Pflüger's method employed by Silvette and Britton (1932) for amounts of tissue from 0.5 to 1.0 gram. Over 200 fetuses from 30 litters were used in the present study.

Growth of the fetal liver. Figure 1 presents the curve obtained when fetal liver weight was plotted against that of the entire fetus. It was apparent that the liver developed at a rather regular rate when the animals were between 1 and 5 grams in body weight. Determinations of the solid constituents of the liver indicated that an increasing degree of liver hydration occurred as gestation proceeded.

The relation of hepatic weight to that of the fetus is shown in figure 2. It is readily apparent that liver growth practically keeps pace with that of the fetus as a whole during the greater part of the gestation period observed, although a rapid decline in liver weight relative to that of the fetus is indicated during earlier fetal development.

Growth of the placenta. The rate of placental growth was found to be rapid (fig. 3) until about the 3-gram stage of fetal body weight; thereafter there was little further increase in placental weight. Total solid determinations on the rat placenta indicated an increase in placental hydration during the period of rapid growth, as shown in figure 3.

The growth of the rat fetus and that of the placenta were found to be quite dissimilar. The placenta reached approximately its maximum development at the 3-gram stage of gestation, in contrast to the progressive increase in fetal weight until parturition.

Glycogen content of the fetal liver. The fetal liver glycogen increased from approximately 1 per cent in the youngest fetuses examined (about 0.4 gram) to over 6 per cent at term. Figure 4 presents the data graphically. The rise in the fetal liver glycogen was found to be fairly rapid throughout the portion of the gestation period studied. Beyond the 2-gram stage, the fetal liver glycogen concentration was observed to exceed the average maternal level, and at term the fetal value was over twice that found in the mother.

Placental glycogen. In the case of the youngest fetuses examined (about 0.3 gram) the placental glycogen was found to be 1.21 gram per cent. No fetal liver glycogen determinations were possible at this early stage. In

0.4-gram fetuses, however, the liver glycogen was observed to be 1.10 per cent, while the placental level was 1.07 per cent. From these values it was apparent (see fig. 4) that the glycogen concentration of the fetal liver first exceeded that of the placenta at some time between the 0.3- and 0.4-gram stages. It seems probable that this "cross-over" point represents the beginning of adult hepatic function, as indicated above.

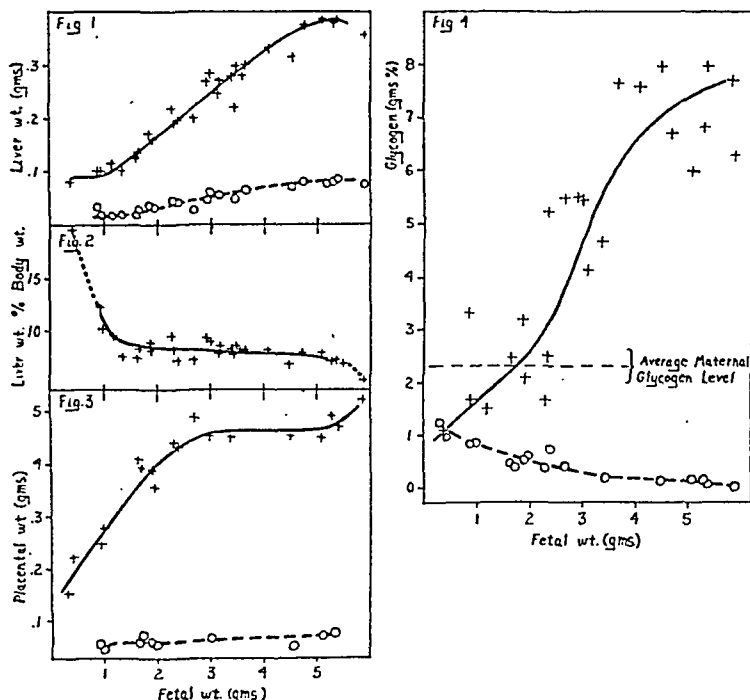


Fig. 1. Growth of the fetal liver showing wet weight (+, solid line) and total solid (O, broken line) determinations.

Fig. 2. Curve showing the percentage of fetal body weight represented by the liver.

Fig. 3. Graphic representation of placental growth; + (solid line) = wet weight; O (broken line) = total solid.

Fig. 4. Glycogen content of the fetal liver and placenta; + (solid line) = fetal liver glycogen; O (broken line) = placental glycogen.

The placental glycogen thereafter fell steadily until term, when it was found to be 0.05 gram per cent. If the "cross-over" point be expressed in percentage of the entire period of gestation, it may be calculated, by employing the data of Angulo (1932), that the assumption of adult glycogenic function by the fetal liver of the rat occurs after the elapse of 75 per cent of the total gestation period. It is of interest to compare this figure with the calculations of Needham (vol. ii, p. 1024), who found the corresponding point to occur at the end of 82 and 91 per cent of the total developmental period in the chick and the rabbit respectively.

It is the contention of Huggett (1928) that, in the rabbit, the maternal portion of the placenta acts in the nature of a "reserve" supply of glycogen for the developing fetus. In his experiments, it was observed that reduction of the maternal liver glycogen, or its enhancement by carbohydrate feeding, produced no effect on the placental glycogen content. Furthermore, Britton (1930) found that the fetal blood sugar level (cat) might remain within normal limits even during insulin-induced hypoglycemic convulsions in the mother.

Observations made on maternal and placental glycogenic levels in the course of the present investigation are in agreement with the above contentions, the maternal liver glycogen varying within wide limits (1.63 . . . 5.3 grams per cent) although the placental glycogen values remained relatively constant.

The present study was undertaken with the view of establishing normal glycogen values to serve as a basis for further observations on materno-fetal carbohydrate metabolism as related particularly to cortico-adrenal function.

SUMMARY

The rate of growth of the liver and placenta of the fetal rat during the latter third of the gestation period has been determined.

A progressive hydration of the fetal liver was evident throughout the period of development studied, the placental water content remaining, however, relatively constant from about the 3-gram stage until term.

The rate of growth of the fetus as a whole exceeds that of the liver until a body weight of slightly over 1 gram is attained, after which general bodily growth and that of the liver progress at relatively the same rate.

The placenta of the rat was found to increase rapidly in size until the fetus attained a body weight of about 3 grams, after which no readily demonstrable change occurred.

At a fetal weight of approximately 0.3 gram, the fetal liver glycogen concentration exceeded that of the placenta. This point of development probably represents the beginning of adult glycogenic function in the liver of the rat.

The placental glycogen concentration fell at a relatively constant rate from the earliest stages studied until birth, although wide variations were recorded in the maternal liver glycogen. This observation supports the contention that the placental glycogen content is relatively unaffected by varying carbohydrate concentrations in the mother.

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A COMPARATIVE STUDY OF SYMPATHIN AND ADRENINE

W. B. CANNON AND A. ROSENBLUETH

From the Laboratories of Physiology in the Harvard Medical School

Received for publication March 14, 1935

In previous communications from this Laboratory it has been shown that sympathin, i.e., the sympathomimetic substance which diffuses into the blood stream when sympathetic nerves supplying autonomic effectors are stimulated, differs from adrenaline (Cannon and Rosenblueth, 1933; Rosenblueth and Morison, 1934; Cannon and Rosenblueth, 1935) and that sympathin from different sources may have different properties (Cannon and Rosenblueth, 1933). These facts led us (1933) to postulate the existence of two sympathins, one possessing exclusively excitatory effects (sympathin E), the other, exclusively inhibitory action (sympathin I).

This view has been contested by Bacq (1934) who points out what he considers weak aspects in our theory and offers alternative hypotheses to account for the data. We believe that a comparative survey of the action of adrenaline and of sympathins from several sources on several indicators should aid in clarifying these conflicting suggestions. Such is the purpose of the present study. We shall first present what may appear to be heterogeneous experimental results, but they will find a natural place in the tables in which we shall summarize the data that are pertinent to a general discussion of the problem.

RESULTS. *The influence of sympathin on the blood pressure.* Stimulation of the cardio-accelerator nerves (c.a.) in cats under dial anesthesia, or spinal and curarized, leads to slight or no changes of blood pressure, attributable to cardiac acceleration (fig. 1A). These changes subside shortly after cessation of the stimulus. If cocaine (8 mgm. per kgm.) is injected intravenously an initial rise of blood pressure, induced by stimulation of the c.a., is succeeded by a delayed and prolonged further rise (fig. 2A). This delayed response is attributable to cardiac sympathin, for its duration and maximal effects outlast the stimulus period and its time relations are closely parallel to those of the contraction of the denervated nictitating membrane used as an indicator of sympathin.

After ergotoxine (2 to 4 mgm. per kilogram), as shown by Dale (1906), adrenaline causes a fall of blood pressure. C.a. stimulation may likewise induce a fall (fig. 1B), explainable as due to a decreased output of the heart in consequence of the acceleration. After ergotoxine and cocaine, adrenaline

produces a fall, while cardiac sympathin elicits a delayed rise of blood pressure (fig. 1C). Thus cardiac sympathin is similar to hepatic sympathin (Cannon and Rosenblueth, 1933) and differs from adrenaline.



Fig. 1. Dial. Vagi cut. Effects of injecting 0.5 cc. adrenaline (1:100,000) and of stimulating the right cardio-accelerator nerves (coil distance, 12 cm.). The heart rate increased in all instances. In this and the succeeding figures the time signal records 30-second intervals.

A. Before ergotoxine and cocaine. B. After ergotoxine (4 mgm. per kgm.). C. After cocaine (7 mgm. per kgm.).

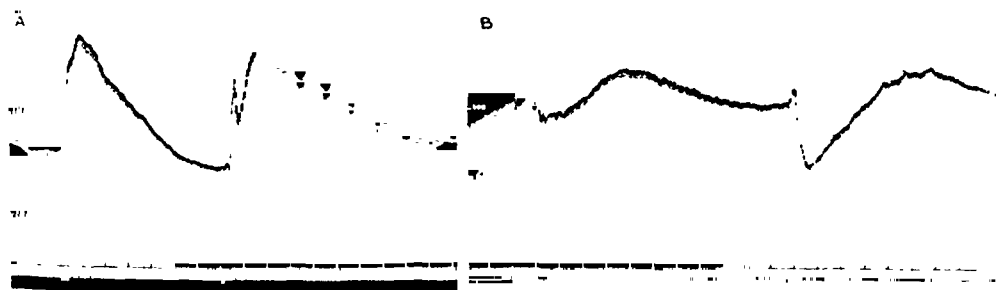


Fig. 2. Dial. Vagi cut. Cocaine (8 mgm. per kgm.). Effects of stimulating the right cardio-accelerator nerves and of injecting 0.3 cc. adrenaline (1:100,000).

A. Before yohimbine. Cardio-accelerators stimulated for 10 seconds; coil distance, 10 cm. B. After yohimbine (3 mgm. per kgm.). Cardio-accelerators stimulated for 30 seconds; coil distance, 8 cm.

Yohimbine (3 mgm. per kilogram) is also capable of differentiating sympathin from adrenaline. After yohimbine adrenaline yields a fall of blood pressure (Hamet, 1925), while sympathin induces a rise, as illustrated in figures 2B, and 3. Injection of both yohimbine and ergotoxine fails also to abolish the rise of blood pressure produced by sympathin (fig. 4).

The source of sympathin is of importance in these observations after

ergotoxine or yohimbine. When sympathetic nerves inducing predominantly or exclusively excitatory effects are stimulated, such as the cardiac or the hepatic nerves, the rises described above ensue. Stimulation of mixed sympathetic nerves, such as the superior mesenteric or the hypogastrics, which induce both excitatory (vasoconstriction) and inhibitory (relaxation of the g.i. tract, the bladder and the non-pregnant uterus) responses, may produce a fall of blood pressure, as does adrenine (cf. Rosenblueth and Cannon, 1935). These falls are more readily obtained after large doses of ergotoxine or in unanesthetized spinal animals, in which the constrictor influence of adrenine is reversed by smaller doses of ergotoxine, than in animals under dial anesthesia (cf. Cannon and Rosenblueth, 1933).

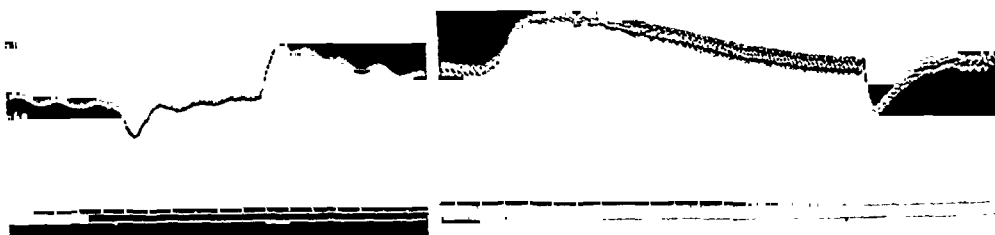


Fig. 3

Fig. 4

Fig. 3. Dial, cocaine (8 mgm. per kgm.) and yohimbine (3 mgm. per kgm.). Adrenals tied off. Hepatic nerves cut. Effects of injecting 0.1 cc. adrenine (1:100,000) and of stimulating the right splanchnic (coil distance, 6 cm.).

Fig. 4. Dial, cocaine (8 mgm. per kgm.), yohimbine (3 mgm. per kgm.) and ergotoxine (2 mgm. per kgm.). Adrenals tied off. Effects of stimulating the hepatic nerves (coil distance, 6 cm.) and of injecting 0.3 cc. adrenine (1:100,000).

The effects of sympathin on the pregnant uterus. Whether sympathin is derived from cardiac, hepatic or gastro-intestinal sources it induces a contraction of the cat's pregnant uterus, as does adrenine. Figure 5 illustrates these responses.

The influence of acetylcholine and atropine on the non-pregnant uterus. Adrenine is known to relax the cat's non-pregnant uterus. Sympathin from certain sources (gastro-intestinal, cardio-pulmonary) has been shown to induce a similar relaxation, while that of hepatic origin fails to evoke this relaxation (Cannon and Rosenblueth, 1933). Acetylcholine in doses sufficient to elicit marked blood-pressure falls is without influence on the non-pregnant uterus (fig. 6A), even after eserine. Large doses after atropine are likewise ineffective (fig. 6B). Adrenine, on the other hand, is still active after atropine (fig. 6B), and so is sympathin from a distant source or that produced locally on stimulation of the hypogastric nerves (fig. 7).

Summary of the effects of sympathins from different sources and of adrenaline on various indicators. We have now at hand data concerning the responses of a considerable variety of organs to sympathin and adrenaline, that reveal

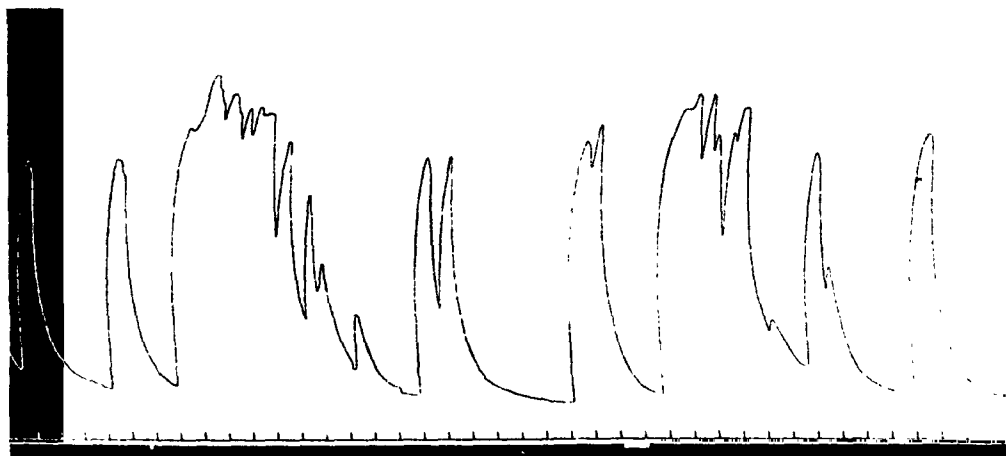


Fig. 5. Spinal. Curare. Cocaine. Record of the pregnant uterus. Effects of injecting 0.2 cc. adrenaline (1:200,000) and of stimulating the hepatic nerves (coil distance, 6 cm.).

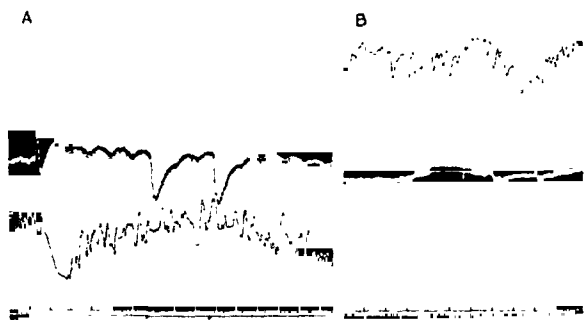


Fig. 6

Fig. 6A. Spinal. Curare. Cocaine. Upper record, blood pressure. Lower record, non-pregnant uterus. Effects of injecting 0.1 cc. adrenaline (1:100,000), 0.05 and 0.1 cc. acetylcholine (1:2,000,000).

B. Spinal. Curare. Atropine (1 mgm. per kgm.). Upper record, non-pregnant uterus. Lower record, blood pressure. Effects of injecting 0.1 cc. acetylcholine (1:100,000) and 0.1 cc. adrenaline (1:100,000).

Fig. 7. Spinal. Curare. Atropine (1 mgm. per kgm.). Response of the non-pregnant uterus to stimulation of the hypogastric nerves (coil distance, 7 cm.).

significant relations. These data are presented in tables 1 and 2. Other sources of sympathin than those listed have been studied (e.g., the hypogastric nerves, the superior cervical sympathetic); and other indicators have been observed (e.g., the retractor penis, the submaxillary gland).

The data are, however, not sufficiently extensive to be included in these tables. It is of interest to note that cocaine increases *all* the effects mentioned in the tables.

In the tables are represented sources of sympathin E and I—the gastro-intestinal tract and the regions innervated by the lower abdominal sympathetic chains and the cardio-pulmonary nerves; and a source of sympathin E alone—the area of distribution of the hepatic nerves (cf. Cannon and

TABLE 1

Comparison of the effects of sympathin and adrenine on various indicators (in the cat), and under various experimental conditions

Double signs (++) or (--) signify greater effects than single signs. G.I. indicates the gastro-intestinal tract; L.a.s., the lower abdominal sympathetic distribution; C.a., the cardio-accelerator distribution; and H.ns., the hepatic nerves.

INDICATORS	ADRENINE	SYMPATHIN			
		G.I.	L.a.s.	C.a.	H.ns.
Heart rate	+	+	+		+
Blood pressure	Small doses -	+	+	++	++
	Large doses +				
Leg vessels	Small doses -	+			++
	Large doses +				
Nictitating membrane...	+	+	+	+	+
Pregnant uterus	+			+	+
Non-pregnant uterus...	-, or - → +	-, or - → +		-, or 0	0
Pupil	++			±	±

TABLE 2

After ergotoxine or yohimbine (same abbreviations as in table 1)

INDICATORS	ADRENINE	SYMPATHIN			
		G.I.	L.a.s.	C.a.	H.ns.
Heart rate	+	+	+	+	+
Blood pressure	-	- or +	- or +	+	++

Rosenblueth, 1933). Also are represented indicators having both a contractile and an inhibitory sympathetic supply (e.g., leg vessels) or only a single supply, contractile (e.g., nictitating membrane) or inhibitory (e.g., non-pregnant uterus). Examination shows that strict concordance between the actions of sympathin and adrenine obtains in the denervated heart, nictitating membrane and pregnant uterus—structures which have single sympathetic (excitatory) innervation. Where there is a dual sympathetic supply—the general arterial system, represented by “blood

pressure" and "leg vessels"—small and large doses of adrenaline have different effects, which theoretically might be duplicated if we could evoke sympathin I separate from sympathin E. Since that has not yet been achieved, however, there is here some discrepancy between adrenaline and sympathin. The most marked discrepancy between them is found in their effects on the non-pregnant uterus, which has a single sympathetic (inhibitory) supply. If sympathin I should reach this uterus it would have the relaxing influence of adrenaline itself; but sympathin E would have no effect. Sympathin from a dual source, therefore, acts like adrenaline, while sympathin from a single (excitatory) source is inactive. It is noteworthy that in comparison with all other indicators the iris, with its antagonistic muscles, stands quite apart (see Cannon and Rosenblueth, 1935).

DISCUSSION. *Important differences between sympathin and adrenaline.* In recent literature on the mediation of sympathetic nerve impulses the effects similar to those produced by adrenaline have been designated "adren-ergic" (Dale, 1934). In suggesting this term Dale was careful to disclaim any intention to prejudge the nature of the chemical mediator. Because the word *adrenergic* is likely to suggest, however, that sympathin and adrenaline are the same, and because the view has been advanced that they are nearly if not quite identical (cf. Bacq, 1933), we wish to summarize briefly here the evidence that they are different. 1. Sympathin E causes a rise of blood pressure after ergotoxine or yohimbine, adrenaline causes a fall (figs. 1, 2, 3 and 4; table 2). 2. Sympathin E is ineffective on the non-pregnant cat uterus, adrenaline causes relaxation (Cannon and Rosenblueth, 1933). 3. Sympathin E stimulates the nictitating membrane, but does not cause dilatation of the pupil; adrenaline induces both changes (Cannon and Rosenblueth, 1935). 4. The summed influence of sympathin E from two sources is greater than the summed influence of two equivalent doses of adrenaline (Rosenblueth and Morison, 1934). Until these observed facts are set aside, or proved of no significance, sympathin E should not be confused with adrenaline.

Sympathin I is not acetylcholine. The existence of sympathetic cholin-ergic fibers (Dale and Feldberg, 1934; Bülbring and Burn, 1934; Rosenblueth and Cannon, 1935) might suggest that sympathin I could be acetylcholine of sympathetic origin while sympathin E could be an adrenaline-like substance with only excitatory action. In the paper mentioned we have given reasons for regarding the fall of blood pressure which may occur on stimulation of sympathetic nerves after ergotoxine and atropine as due to a non-cholinergic sympathin I. The data on the non-pregnant uterus are furthermore conclusive in this respect; acetylcholine does not induce relaxation, either before or after atropine (fig. 6), while adrenaline does induce it (fig. 6), as does stimulation of the hypogastri-ics, whether atropine be present or not (fig. 7). We therefore conclude that there is a sympathin

I—i.e., an “adrenergic” product of sympathetic nerve stimulation—which differs from acetylcholine and does not act by liberating acetylcholine, but possesses relaxing properties of its own.

Defense of the concept of sympathins E and I. Three more or less different substitutes for this concept have been put forward by Bacq (1934).

1. The first alternative (p. 480) is that the sympathetic chemical mediator, M or sympathin, is equivalent to adrenaline, that it oxidizes rapidly (presumably on its way from source to indicator, for Bacq stresses the long interval—2 minutes—between the start of stimulation and the start of response), and that by partial oxidation it might lose its inhibitory power. It would then become, in our view, equivalent to sympathin E, excitatory only. This hypothesis does not accord with the fact that sympathin E is obtained from sources (liver, heart) which yield it in large amounts—which would probably be more slowly oxidized than small amounts—and so promptly that the positive effects are produced, not in minutes, but in a few seconds. Thus far sympathin I has been demonstrated most strikingly when derived from the alimentary canal, a source which apparently yields small amounts and from which it passes slowly to the test object—just the condition which, according to Bacq, would be most unfavorable for its appearance. Furthermore, if in partial oxidation adrenaline loses its inhibitory action, one would expect that small doses, quickly oxidized, would be purely excitatory; in fact, on blood pressure they have a depressor effect. And finally, if sympathin is the same as adrenaline it should act like adrenaline after ergotoxine on arterial pressure (cf. Cannon and Rosenblueth, 1933), and on the iris (Cannon and Rosenblueth, 1935), but it does not.

2. The second alternative offered by Bacq (p. 480) involves the postulation of two mediating substances: adrenaline where the sympathetic has inhibitory effects, and noradrenaline (i.e., non-methylated adrenaline) where excitatory. Note that demethylation would be necessary before injected adrenaline could exert its excitatory action. And if that process should occur routinely, it would probably occur more rapidly with small than with large amounts. The depressor action of small doses, therefore, would not harmonize with this second suggestion. Also it fails when adrenaline and sympathin are compared in their effects on the iris.

3. The third possibility considered by Bacq (p. 481) is that potassium ions liberated when the sympathetic is stimulated would accentuate the excitatory and lessen the inhibitory influence of sympathin. Bacq and Rosenblueth (1934) have shown, however, that when potassium ions are injected they induce contraction of the non-pregnant cat uterus if the adrenals have previously been excluded, but relaxation if not excluded. The relaxation, therefore, is due to medulliadrenal secretion (evoked by the potassium) which may be regarded as overwhelming the contractile action of potassium. If sympathin E were adrenaline *plus* potassium ions

it might be expected to make the uterus relax to a marked degree, due not only to the adrenine present in it, but also to the adrenine which the potassium ions evoke from the adrenals. But we know that when either the hepatic nerves or cardio-accelerators are stimulated, in such manner as to produce sympathin E, the nictitating membrane is made to contract and the cat's non-pregnant uterus is not made to relax (Cannon and Rosenblueth, 1933). Bacq's third suggestion, therefore, appears, like the others, to meet insurmountable obstacles.

Another consideration of some importance is that Bacq's three suggestions fail to explain simply and reasonably the *change* in the responses of the cat uterus to adrenine and sympathin, from relaxation when non-pregnant to contraction during pregnancy. The view which we are defending would explain the change as a conversion of the receptor I into E.

Admittedly the existence of sympathin I, apart from sympathin E, has not been demonstrated—a fact explicable by failure to isolate in the organism a purely inhibitory sympathetic nerve supply because vasoconstrictor (excitatory) fibers seem to be everywhere present. Even though sympathin I, different from adrenine, has been postulated on only indirect evidence, however, it offers the sole hypothesis which accounts for inhibition by sympathetic impulses and adrenine. And furthermore, if the existence of sympathin E is granted—for which there is direct evidence—the inhibitory agent, sympathin I, cannot, we are convinced, be pure adrenine. In that case stimulation of a mixed source (e.g., splanchnic stimulation of the gastro-intestinal tract) would yield sympathin E from the contracted and adrenine from the inhibited smooth muscle. But adrenine is both excitatory and inhibitory. In consequence, the mixture would always have an excess of excitatory effect if its inhibitory effect should be matched with adrenine—i.e., if the relaxation of the non-pregnant cat uterus from splanchnic stimulation were equaled by a certain amount of adrenine, that amount could not produce the corresponding contraction of the nictitating membrane, for that would have resulted from sympathin E *in addition to the adrenine*. But it is quite possible to reproduce quantitatively the effects of sympathin, + and –, by the injection of adrenine (see Cannon and Rosenblueth, 1933; also unpublished observations by Bacq, Cannon and Rosenblueth). We therefore conclude that if the existence of the excitatory sympathin E is admitted, the inhibitory chemical mediator is not adrenine, and must be a humoral agent arising from sympathetic stimulation and having special inhibitory power. That would be a good definition of sympathin I.

SUMMARY

The effects of sympathin from various sources—gastro-intestinal tract, hind limbs and tail, heart and lungs, and liver (in cats)—are compared with

the effects of adrenine on the following indicators: heart rate, blood pressure, leg vessels, nictitating membrane, pregnant and non-pregnant uterus, and pupil (table 1). A similar comparison is made of the effects of sympathin and adrenine on the heart rate and blood pressure after yohimbine or ergotoxine (table 2). The data contained in tables 1 and 2 are partly drawn from previous publications and have been completed by the results reported in the present study (figs. 1 to 7). Discussion of these data leads to the following conclusions: a, sympathin differs from adrenine (p. 273); b, there are two sympathins, one excitatory (E), the other inhibitory (I) (p. 274); and c, sympathin I is not acetylcholine (p. 273).

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THE EQUATION OF THE VOLTAGE-CAPACITY CURVE FOR THE EXCITATION OF THE SCIATIC NERVE OF *RANA PIPIENS*

H. A. BLAIR

From the Department of Physiology, School of Medicine and Dentistry, The University of Rochester, Rochester, N. Y.

Received for publication March 22, 1935

Considerable evidence has been presented of the validity of a certain description, in the form of equations, of the processes of excitation in response to electrical stimuli of various kinds (1932-35). In no case, however, has a large number of excitation curves obtained with one type of stimulus been considered in order to demonstrate the presence or absence of systematic divergences between the data and the equation devised to represent them. It is the present purpose to consider a group of voltage-capacity curves from this point of view.

There is sufficient evidence that any divergences which may exist with the muscles and nerves of the frog, at least, are small, so that as regards the use of the scheme developed as an aid in planning and correlating experiments, the question of whether or not they are systematic is of relatively little importance. It is of importance, however, to consider any systematic divergences when the problem arises of interpreting in physico-chemical terms the equations of the phenomenological scheme, particularly when it is necessary to choose between two or more modes of representation which appear about equally probable and relate the data about equally well.

Another purpose served by determining the equation of any type of stimulus accurately is the establishment of a criterion whereby any hypothesis concerning the excitatory process may be tested easily. For if an equation exists, which has been shown to represent a certain body of data with a certain degree of accuracy, then any hypothesis of probable validity must be representable by an equation which reduces to the first with at least equal accuracy; and in general it is much easier to compare two equations than it is to test one by applying it to a large body of data.

The present purpose is to show the accuracy of representation of a large number of voltage-capacity data from the stimulation of the sciatic nerve of *Rana pipiens* by the equation,

$$\frac{V}{R} = (crk)^{\frac{1}{\alpha k - 1}} \quad (1)$$

where V is the peak voltage of the stimulus, R is the rheobase, c and r are the capacity and resistance, respectively, of the circuit, and k is a constant.

This equation is the solution (1932c) of the differential equation,

$$\frac{dp}{dt} = KV - kp \quad (2)$$

where p is the excitatory process and K is a constant, using the conditions that p is initially zero and is adequate at its maximum when its maximum value is equal to $h \pm \alpha V'$, h and α being constants, and V' being the voltage at the time p is a maximum. Appropriate solutions of this equation with the same boundary conditions have been shown to be consistent for direct currents, linearly rising currents and condenser discharges as stimuli (1935a, b). With condenser stimuli the solution of equation 2 is the same, i.e., equation 1 is the same, whether the threshold is $h \pm \alpha V'$, or simply h . Consequently k is the only parameter whose value is obtainable by means of equation 1.

EXPERIMENTAL METHOD. All the data were obtained from sciatic-gastrocnemius preparations of *Rana pipiens* which had been kept in cold Ringer's solution from one to several days after dissection. The nerve was suspended in air on the electrodes, which were silver wires 0.5 mm. in diameter lightly covered with the chloride. The electrode separation was from 1 to 2 cm. The stimulating circuit consisted of a battery of 135 volts in series with the condenser and a non-inductive resistance of 50,000 ohms. Part of this resistance was used as a potentiometer from which the stimuli were derived. The resistance of the potentiometer between the leads never exceeded about 1 per cent of the resistance of the tissue. The charging current was used to stimulate and the condenser was discharged between stimuli by short-circuiting. The condensers were of high quality with mica dielectric. The temperatures, which covered nearly the whole physiological range, were maintained constant to about 0.2°C. during the taking of each set of data. The time required to obtain each set averaged about 6 minutes. Each datum is a single determination. The rheobase was measured initially and finally.

DATA AND METHOD OF CALCULATION. In table 1 are given 36 sets of data. These were selected from about 50 by eliminating first those in which the final rheobase differed from the first by more than about 2 per cent. This left 45 sets which were calculated. The 36 most consistent of these were then selected for presentation. Of the remainder, five had rheobases less than 40 units and the consequent inaccuracy of reading made the plotted curves irregular and the evaluation of k difficult. The other four curves had the largest single divergences.

In the table, the first column gives the capacities of the condensers in

TABLE 1
VOLTAGE CAPACITY CURVES AS MEASURED
AND CALCULATED ACCORDING TO EQUATION 1

	A			B			C			D			E			F		
	V OBS.	V CAL.	V/V ₀	V OBS.	V CAL.	V/V ₀	V OBS.	V CAL.	V/V ₀	V OBS.	V CAL.	V/V ₀	V OBS.	V CAL.	V/V ₀	V OBS.	V CAL.	V/V ₀
6.0	63	63		42	41		77	77		43	43		37	37		64	64	
1.0	63	67	1.06	45	43	0.96	79	80		43	45	1.05	38	39	1.03	65	65	
0.5	68	70	1.03	47	45	0.96	83	90	1.08	43	46	1.07	40	40		68	69	1.02
0.1	86	87		62	56	0.90	99	100		54	54		48	47	0.98	80	80	
0.05	104	104		70	67	0.96	117	122	1.04	62	62		55	54	0.98	91	91	
0.02	145	146		100	93	0.93	157	156		78	80	1.03	72	70	0.97	120	119	
0.01	211	217	1.03	130	132	1.02	212	217	1.02	105	105	0.98	95	91	0.96	160	159	
0.008	237	236		160	150	0.94	240	241		118	122	1.03	104	106	1.02	175	172	0.98
0.004	372	371		230	234	1.02	361	363		175	177		154	154		260	260	
0.003	458	458		285	288		442	444		213	212		190	185	0.97	310	314	1.02
0.002	624	623		392	392		580	592	1.02	283	282		249	246		413	415	
0.001	1115	1120		636	688	1.08	1032	1029		478	473		428	414	0.97	700	705	
6.0	63		K = 1390	41		K = 1460	77		K = 1920	43		K = 2400	38		K = 2400	64		K = 2430
6.0	47	47		48	47		37	37		41	41		51	50		44	44	
1.0	47	49	1.04	47	49	1.04	38	38		41	42	1.02	51	51		44	45	1.02
0.5	49	50	1.02	49	50	1.02	39	39		42	43	1.02	51	53	1.04	45	46	1.02
0.1	58	58		57	57		47	45	0.96	50	49	0.98	59	60	1.02	51	52	1.02
0.05	64	66	1.03	65	65		52	50	0.96	57	55	0.96	66	66		58	58	
0.02	85	85		84	82		67	63	0.94	72	70	0.97	86	83	0.97	72	72	
0.01	113	113		107	107		84	82	0.98	92	91		112	106	0.95	92	92	
0.008	122	124	1.02	119	119		107	107	0.98	101	97	0.98	119	117	0.98	100	100	
0.004	178	182	1.02	170	172		134	129	0.96	146	143	0.98	163	164		146	140	0.96
0.003	210	229	1.09	203	206	1.02	157	154		172	170		194	194		164	166	1.02
0.002	281	288	1.03	260	269	1.03	204	201		222	222		252	253		215	215	
0.001	482	488		448	452		334	362	1.08	364	401	1.10	412	414		351	352	
6.0	47		K = 2610	47		K = 2890	37		K = 3140	41		K = 3140	50		K = 3480	45		K = 3660
6.0	141	142		39	39		71	70		56	56		40	40		72	71	
1.0	146	149	1.02	41	41	1.03	72	72		57	57	1.02	41	41	1.02	72	73	
0.5	146	149	1.02	41	41		72	73		57	59	1.03	41	42	1.02	73	74	
0.1	168	168		46	46		80	82	1.02	65	66	1.02	46	47	1.02	82	83	
0.05	190	186	0.98	52	51	0.98	93	88	0.95	73	73		52	51	0.98	91	91	
0.02	237	230	0.97	65	63	0.97	115	112	0.97	92	90	0.98	63	63		110	100	0.91
0.01	302	293	0.97	80	80		144	140	0.97	114	113		81	79	0.98	138	140	
0.008	322	321		89	87	0.98	162	155	0.96	127	124	0.97	89	86	0.97	154	153	
0.004	460	450	0.98	120	122	1.02	225	217	0.96	177	174	0.98	124	120	0.97	209	212	
0.003	535	531		140	144	1.03	268	255	0.95	212	203	0.96	145	140	0.97	242	248	1.02
0.002	695	672	0.97	180	185	1.03	336	328	0.97	263	255	0.97	181	180		307	318	1.04
0.001	1125	1120		304	303		533	533		428	425		288	290		517	517	
6.0	143		K = 3720	39		K = 3800	70		K = 3900	58		K = 3930	40		K = 4150	771		K = 4170
6.0	62	62		77	77		72	71		67	66	0.98	82	80		63	63	
1.0	62	63	1.02	77	79	1.02	72	73		65	68	1.05	82	81		63	64	1.02
0.5	65	65	1.02	79	80		73	74		65	69	1.06	84	84	1.02	65	66	1.02
0.1	71	72		87	89	1.02	82	82		75	76		94	92	0.98	73	73	
0.05	78	79		99	98		90	91		79	83	1.05	104	102	0.98	81	79	0.97
0.02	96	97		120	119		112	109	0.97	104	100	0.96	127	122	0.96	100	96	0.96
0.01	123	122		154	149	0.97	138	136		129	125	0.97	155	151	0.97	124	119	0.96
0.008	133	133		163	163		153	148	0.97	140	136	0.97	167	165		132	130	
0.004	181	185	1.02	223	222		205	206		187	189		228	228		180	180	
0.003	215	216		260	262		244	239	0.98	225	218	0.98	261	264		209	208	
0.002	275	277		329	338	1.03	306	306		272	276		329	335	1.02	257	264	
0.001	420	446	1.06	514	542	1.05	488	492		433	442	1.02	534	537		416	422	
6.0	63		K = 4200	77		K = 4360	71		K = 4450	67		K = 4660	80		K = 4660	63		K = 4660
6.0	44	44		71	70		69	68		55	54		52	52		56	54	
1.0	44	45	1.02	71	72		70	70		54	55	1.02	52	53	1.02	55	55	
0.5	45	46	1.02	71	73	1.03	70	71		55	56	1.02	54	54		55	55	1.02
0.1	50	51	1.02	79	80		78	78		60	62	1.03	59	59		61	62	1.02
0.05	56	55	0.98	88	86	0.98	87	82	0.94	66	65	0.98	66	65	0.98	65	67	1.03
0.02	69	67	0.97	107	105	0.98	105	104		80	81	1.02	80	78	0.98	80	80	
0.01	84	83		134	130	0.97	129	126	0.98	100	100		97	95	0.98	98	98	
0.008	90	90		143	143		144	137	0.95	109	108		107	103	0.96	106	107	
0.004	122	122		192	188	0.98	193	171	0.89	142	135	0.95	144	141	0.98	140	146	1.04
0.003	144	144		226	225		223	217	0.97	170	172		172	163	0.95	160	167	1.04
0.002	181	182		287	286		274	275		213	215		218	205	0.94	210	212	
0.001	283	290	1.02	455	453		432	435		337	346	1.03	328	326		315	316	1.07
6.0	44		K = 4730	70		K = 4860	68		K = 4950	54		K = 4970	52		K = 5090	54		K = 5150
6.0	61	61		58	57		56	56		87	87		86	86		95	95	
1.0	61	62	1.02	58	58		56	57	1.02	87	87		87	87		96	96	
0.5	61	63	1.03	60	59	0.98	57	58	1.02	88	89		87	88		96	97	
0.1	69	70		65	63	0.97	62	62		95	94		94	93		99	101	1.02
0.05	75	75		72	68	0.94	69	67	0.97	104	99	0.95	101	97	0.96	104	106	1.02
0.02	92	90	0.98	84	80	0.95	83	78	0.94	117	112	0.96	113	109	0.96	115	117	1.02
0.01	114	109	0.96	100	95	0.95	100	94	0.94	127	128		130	125	0.96	132	133	
0.008	123	118	0.96	110	105	0.95	107	102	0.95	145	137	0.94	139	132	0.95	138	140	
0.004	163	160	0.98	142	135	0.95	138	133	0.96	178	171	0.96	166	164		168	172	1.02
0.003	188	185	0.98	161	156	0.97	159	153	0.96	204	192	0.94	193	184	0.95	187	191	1.02
0.002	236	234		195	193		194	189	0.97	240	232	0.97	220	219		220	227	1.03
0.001	365	368		293	297		290	292		355	338	0.95	308	317	1.03	300	321	1.07
6.0	61		K = 5380	57		K = 6650	56		K = 6650	87		K = 10500	86		K = 11350	95		K = 13200

C IN MICROFARADS; UNIT $V = 27 \times 10^{-4}$ VOLT; RESISTANCE = 50,000 OHMS.

microfarads. The second gives the measured voltages of the stimuli while the third contains the voltages calculated according to equation 1. In the fourth column are the ratios of the calculated to the measured voltages. The remaining columns in each row, in sets of three, are the same as the second, third and fourth. The numbers 1, 2, etc., and the letters A, B, etc., identify the separate curves. The 6 microfarad reading is taken as the rheobase.

The sets of data are arranged in order of increasing k values, that of the least being 1390 and the greatest 13,200. The temperatures at which the readings were made increased in about the same order, those in row 1 having been from 6 to 11°C., in row 2 from 9 to 16°C., in row 3 from 11 to 19°C., while most of the remainder were from 20 to 28°C. The data are representative, therefore, of the physiological temperature range and as a consequence of the normal range of the values of k , for the most easily stimulated nerve elements of the trunk.

The extent of the curves, i.e., the number of rheobases to which they extend, is variable because no capacity less than 0.001 microfarad was used. Consequently curves of large k are shorter than those with small, so that the last set of data goes to only three rheobases, while the first goes to about seventeen. The intermediate sets extend on the average to about eight rheobases.

The method of calculation has been given before (1932c, 1934). The constant k was determined as usual from the linear relation,

$$cr \log \frac{V}{R} = \frac{1}{k} \left\{ \log \frac{crV}{R} + \log k \right\} \quad (3)$$

which is the same as equation 1. In calculating the quantities $cr \log V/R$ and $\log crV/R$ the smallest measured rheobase was used if the initial and final values were not equal. The line determined by these quantities should, according to equation 3, have a slope of $1/k$ and an intercept on the abscissa, $-\log k$. The mean value of k from these two sources was used to calculate V , the measured values of R and cr being assumed correct. Actually in the method, the quantity k is obtained from the data for the smaller half of the capacities because the line determined using voltages too near the rheobase is too sensitive to small variations.

It will be noted that a systematic divergence is introduced by the method on account of the smallness of the rheobase which is often about 50. This number must always be greater than or equal to the real value which will lie between the measured value and one unit less than the measured value. The measured value of the rheobase, therefore, which has been assumed to be correct actually is always too great by an amount which may be as much as 2 per cent, approximately. On the other hand, the highest voltages are measurable with greater accuracy because they are

in the hundreds. As a consequence of these facts, the quantities V/R of equation 3 will be usually somewhat too small and k , therefore, will be somewhat too large. On account of k , the curvature of the theoretical curve will be somewhat too great although the method makes it match the measured curve at the ends. In all cases, therefore, in which the measured rheobase is too high the calculated voltages will tend to be somewhat too low in the middle of the curve and too high near the rheobase. This divergence should not be great but it will be systematic.

Another feature of the method to be noted also in connection with the rheobase, is that it has been assumed to be correct in finally calculating V . Referring to equation 1, it will be seen that even if k has been correctly determined, and if the right hand of equation 1 has been calculated correctly, an error will occur in V when it is obtained by multiplying the right side of equation 1 by R , if R is too great or too small. The effect of this will be to make all the calculated values of V too great or too small in the same ratio as R is too great or too small. This effect could be adjusted by choosing a theoretical rheobase which gave the best fit, but this was not done in the present case, except that in three instances the mean of the initial and the final rheobases was used instead of the smallest one.

It must be emphasized that these data for the reason given above, and because the temperature was not controlled very closely and because the measurements were not repeated, are probably not as exact as they might be. Any improvement except in regard to temperature would probably require a greater time in taking each curve, but this would not likely be serious as the preparations are very stable. The lack of repetition of measurements, in particular, although saving in time, allows possible occasional large errors in reading the voltage to pass unnoticed, and for this reason it seems certain that data could be obtained whose large errors in measurement were much reduced, and it is altogether likely that the readings near the rheobase could be made somewhat more exact by the use of a finer scale of voltages. The present data have advantages, however, in that they cover a large range of temperatures, are numerous, and are routine measurements not highly selected, so that they will not only test the validity of equation 1 but will indicate also how closely ordinary measurements can be expected to conform to it.

DISCUSSION. It is difficult to form an estimate of the real errors of observation since the tissues are subject to variation. It is indicated by the agreement of the initial and final rheobases, however, that, in the cases given, this variation is usually within the limits ± 2 per cent. The rest of the curve may vary independently of the rheobase, however, particularly as the result of temperature changes, so that the rheobase agreement is not necessarily an adequate index. As regards the apparatus, the series resistance was kept adjusted to 50,000 ohms within ± 0.2 per cent. This

variation and that of the tissue will not be systematic so that in their regard the variations should lie within the range of ± 3 per cent. The shunting of the potentiometer by the tissue may introduce with high voltages a systematic positive error in the readings not exceeding 1 per cent. The condensers were factory standardized to at least ± 2 per cent with the smallest capacities, the remainder being better. Apart then from the systematic instrumental errors which will exist only with the smallest capacities, it may be expected that the bulk of the observations will be correct to ± 3 per cent and the data will be interpreted on this assumption.

It will be seen from table 1 that the measured and calculated voltages are in close agreement. In order to make comparison easier, all the ratios of calculated to measured data which were less than 1.5 per cent greater or less than unity were omitted from the ratio columns. Such divergences may in any case be considered to be within the limit of accuracy of the apparatus and method of calculation. Ratios greater than 1.015 and less than 1.025 are given as 1.02 and so on.

In figure 1 is given a graphical summary of the divergences. The heights of the blocks give the percentage of the observations which fall within different groupings of percentage variation from the theoretical curve. The ranges of the variations, 0, 0-1.5, etc., are given at the tops of the blocks. The positive variations are toward the right, the negative toward the left. It can be verified roughly that 48.4 per cent of all the observations lie within ± 1.5 per cent of the theoretical curve, 69.4 per cent lie within ± 2.5 , and 81.8 per cent lie within ± 3.5 per cent. The mean of the last two values, 75, will give, approximately, the percentage of the data which lie within ± 3 per cent of the curve, which has been estimated above to be about the range of experimental error. This could be improved somewhat by adjusting the rheobases in some cases, for there are 7 curves in which all the divergences are positive or zero, and 4 in which they are negative or zero. It is evident that in these cases the divergences could be made smaller by adjusting the theoretical curve so that the errors were both positive and negative.

In table 2 is given a summary of the divergences referred to corresponding parts of the curves on a scale of rheobases. These parts are taken as those from 1-1.5 rheobases, 1.5-3.0 rheobases, etc., as indicated in the last column of the table. The first column gives the number of positive divergences greater than 1.5 per cent in each category and the second their sum. The third and fourth columns give similarly the negative divergences and their sum. The fifth column contains the numbers of zero divergences (less than 1.5 per cent) while the sixth gives the total number of observations. In the second last column is given the average divergence which is obtained by dividing the sums of the divergences by the total number of observations, each zero divergence being given, arbitrarily, the value unity for this purpose.

It will be seen that each category has one-half or more of its observations with zero divergence except that from 1.5-3.0 rheobases. In this case only three-eighths have zero divergence and many more of these are negative than positive, indicating that there is a systematic tendency

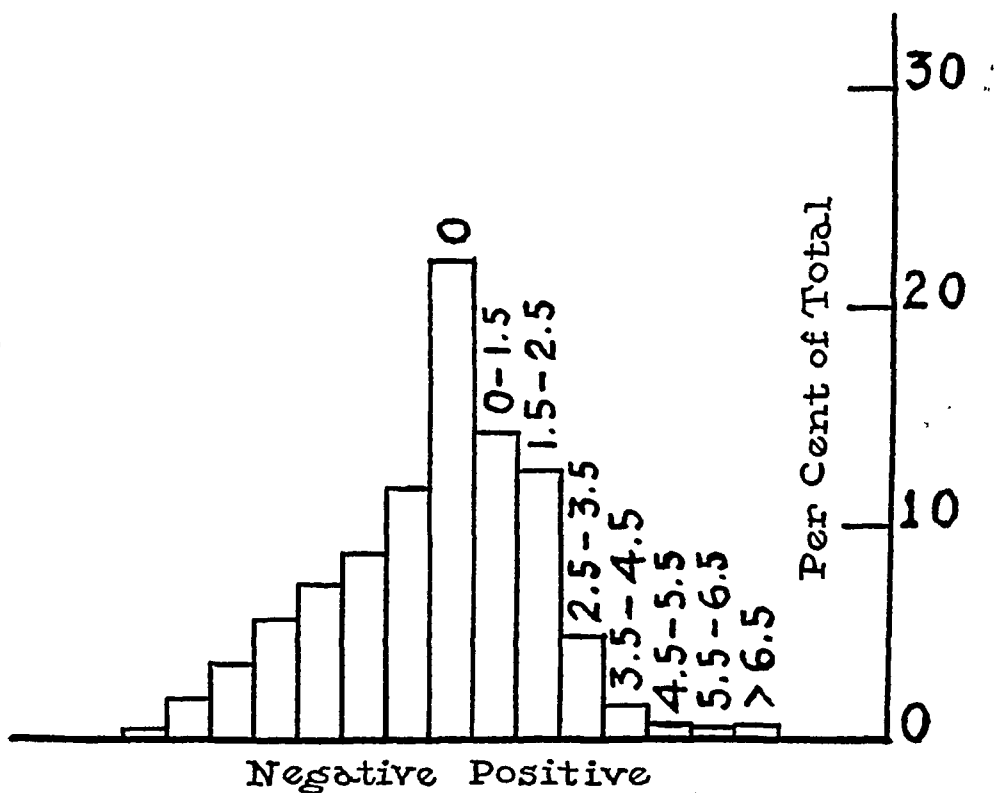


Fig. 1

TABLE 2

The distribution of the divergences related to different parts of the curves on a scale of rheobases

POSITIVE		NEGATIVE		ZERO		TOTAL NUMBER OF OBSERVA- TIONS	AVERAGE DIVERGENCE	RANGE IN RHEOBASES
Num- ber	Sum	Number	Sum	Number	Sum			
57	155	34	109	97	97	186	1.9	1 -1.5
11	33	66	209	45	45	120	2.3	1.5-3.0
20	62	22	70	42	42	83	2.1	3.0-6.0
10	44	1	3	27	27	38	2.0	>6.0

here for the theoretical curve to fall lower than the observed curve. Since all the curves have been treated in the same way, the average divergence permits an estimation of this discrepancy, because the average divergence in any category indicates approximately the average distance at which

any curve representing the observations could lie from the present curve. Therefore, since the average divergence in each group is about 2, even if the groups on either side of that from 1.5–3.0 rheobases had all their divergences of positive sign while that group had all its divergences of negative sign, the average total systematic divergence in these regions could not differ greatly from the arithmetic sum of the averages, i.e., from 4 per cent. Therefore, even if there is real systematic divergence in the curves, i.e., if this apparent one is not accounted for by the fault, mentioned above, in the method, it cannot greatly exceed the limits ± 2 per cent.

The matter may be considered also with regard to the individual curves rather than the group. In table 3 are given the total divergences in each curve. The rows and columns have the same designations as those in table 1. The numbers give the arithmetic sums of the greatest positive and the greatest negative divergences in each curve. It is evident that a fit could be made in each case such that the greatest negative and the greatest positive divergences were equal to each other and therefore to half their

TABLE 3

	A	B	C	D	E	F
1	6a	18	8a	9c	7c	4a
2	9c	4a	16	14b	9c	6b
3	5bc	6a	6	7c	5bc	15c
4	6	8	3a	10	6bc	7a
5	5c	5bc	11b	8c	8	7
6	7c	6	8	6	8c	7

sum as given in the table. It will be seen that there are 27 curves with total divergences of 8 or less, 15 curves with 6 or less, 7 curves with 5 or less, and 3 curves with 4 or less. In each of these groups a fit could be made so that no observation disagreed by more than ± 4 or ± 3 , etc.

Also in table 3, along with the numbers, are the letters *a*, *b* and *c* which mark, respectively, those curves in which at least half the observations in each category of rheobases have zero divergence, in which at least one has zero divergence, and in which at least half diverge by ± 3 or less. These cases include, as can be seen from the summary above, 24 curves. There can be but few, if any, of these in which equation 1 does not fit the observations within the estimated experimental error ± 3 per cent. Of the remaining curves, all but four have total divergences less than 8.

CONCLUSIONS

It appears possible to conclude that equation 1 containing the single arbitrary constant *k* represents the voltage-capacity curves obtained from the stimulation of the sciatic nerve of *Rana pipiens* within or close to the

limits of the experimental error with the present method. A small systematic divergence is apparent at the part of the curve between 1.5 and 3.0 rheobases, but since a divergence of the same kind is introduced by the method of curve fitting employed, the discrepancy, if real, must be less than that indicated. If at any time a choice is necessary between equation 1 and some other which appears to fit equally well it will be necessary to determine the fit of these equations by more elaborate methods, but this can be done on fewer data taken at the most favorable temperature. Until that time, equation 1 may be used to test any hypothesis concerning the excitatory mechanism, for any such hypothesis, if valid, must, when expressed mathematically, give a form which will reduce to equation 1 to within narrow limits. In addition, the local excitatory process may be studied safely by expressing its variation as a function of experimental conditions in terms of the parameter k . For even if equation 1 is not the true representation of the local excitatory mechanism, the constants of the true representation must be expressible in terms of k and the rheobase alone because these two factors, according to equation 1, enable, within close limits, the reproduction of the voltage-capacity curve. They embody, therefore, all the information obtainable from this curve.

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EFFECT OF A DIET POOR IN SALTS UPON THE GROWTH AND COMPOSITION OF THE INCISORS OF THE RAT^{1,2}

MIRIAM F. CLARKE AND ARTHUR H. SMITH

From the Department of Physiological Chemistry, Yale University, New Haven

Received for publication February 18, 1935

According to the newer point of view in nutrition, the skeleton is considered as a reservoir of mineral salts, which can be mobilized from this structure with surprising readiness. Inasmuch as the teeth are in many ways similar to bone, there is a question as to whether these organs likewise represent a labile store of mineral salts. It has been demonstrated that the chemical composition of the teeth of rats is relatively constant in the face of deficiencies such as lack of adequate calcium and vitamin D (8, 21, 9, 10, 20). The incisors, which continue to grow both in young and in adult rats, even though the diet may be lacking in tooth-forming material, are thus different from the bones which, although they may continue to grow, may suffer considerable change in chemical composition.

The present study was undertaken to determine to what extent changes occurred in the teeth of rats kept on a ration in which the mineral salts were reduced to a minimum but which was otherwise adequate; and to discover whether the changes, if they occurred, could be reversed by introducing the lacking elements into the diet. Particular attention has been given to the recovery phase in its chemical aspects. The ration used caused a cessation of growth in the experimental animals. Similar dietary restriction has been shown to bring about a distortion of the body weight to body length ratio due to the persistence of growth of the skeleton (22). Accompanying alteration of chemical composition of the bones has also been described (16).

EXPERIMENTAL. Young, rapidly growing male rats from the strain described by Anderson and Smith (2) were used in this study. They were raised on the stock diet (diet 1) until they weighed 100 grams, at which time their average age was 35 days. No rat was used which was older than 40 days at a body weight of 100 grams. Groups of 10 to 20 rats were then caged separately and offered *ad libitum* the low-salt diet (diet 3, table 1).

¹ Some of the data in this paper are taken from a dissertation presented by Miriam F. Clarke in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Yale University, 1933.

² Aided by a grant from the Research Fund of the Yale University School of Medicine.

At the termination of a 3, 6 or 12 week period, groups were killed, their upper incisors carefully removed and weighed. Moisture content and ash determinations were made, the latter after desiccation at 90°C. and extraction with alcohol and ether. Two to four pairs of teeth were extracted and ashed together and the average weight and per cent of inorganic residue per pair of incisors were calculated (table 3).

Two types of controls were employed: 1, age controls, littermates of the rats on the low-salt ration, but fed adequate synthetic ration (diet 2, table 1) from the time they weighed 100 grams, for the same periods (3, 6 and 12 weeks); and 2, calorie controls, also littermates, fed a diet containing adequate mineral salts, but restricted in calories (diet 4, table 1). The amount fed to each calorie control rat per day was determined by the amount of diet 3 consumed by the low-salt rats, whose food consumption gradually diminished during the experimental period. In equating the

TABLE 1
Composition of the experimental diets

	DIET 2 "ADEQUATE SYNTHETIC"	DIET 3 "LOW-SALT"	DIET 4 "CALORIE CONTROL"
Washed casein (18).....	18	18	16.9
Hydrogenated fat ("Crisco").....	27	27	25.4
Corn dextrin.....	51	55	51.8
Salt mixture (14).....	4	0	5.9

N. B. Each rat received daily food accessories as follows: 5 drops cod liver oil, 1 cc. of an 80 per cent alcoholic extract of wheat germ equivalent to 2 grams of wheat germ, 200 mgm. of dried brewer's yeast.

allowance of food energy, due consideration was taken of the non-caloric portion (mineral matter) in diet 4. The growth curves of these calorie controls were midway between those of the age controls and of the low-salt rats; their retardation in growth was due solely to inadequate energy intake, whereas the low-salt rats suffered two deficiencies, namely, energy-producing and inorganic matter.

Other groups of rats, after subsisting on the salt-poor ration for the above-mentioned periods, were realimented with an adequate synthetic diet (diet 2) given *ad libitum* for various lengths of time as indicated in table 2. The controls for this part of the experiment consisted of 1, age controls, of the same age as the low-salt rats on the day of sacrifice, fed diet 2 throughout, i.e., for 12, 18 or 24 weeks; and 2, calorie controls, fed diet 4 in restricted amounts for 3, 6 or 12 weeks, then diet 2 *ad libitum* for the same time as were the realimented low-salt rats.

At the termination of the above periods, each rat was killed and the teeth treated as previously described.

The casein used in the diets was repeatedly washed with acidulated water in an attempt to remove the inorganic residue (18). The ash of the low-salt diet, determined by the calcium acetate method (12) was 0.44 per cent. Similar determinations on diets 2 and 4 were 2.78 and 3.59 per cent, respectively. The extremely low concentration of the basic ions constituted the chief deficiency in the low-salt diet. The daily intake of individual ions under similar experimental conditions has been discussed (5, 17).

RESULTS. The incisors of the low-salt rats continued to grow during the period when salts were withheld, but at a subnormal and decreasing rate (table 2). The fresh weight of the teeth of the low-salt rats was 92 per cent that of the age controls at 3 weeks, 84 per cent at 6 weeks and 79 per cent at 12 weeks. The weight of the incisors of the calorie controls, on the other hand, never fell lower than 92 per cent of that of the corresponding age controls during the 12 weeks period. These changes in the weights of the teeth of the experimental animals are not in proportion to the relative loss of body weight of the low-salt and of the calorie control rats. The more pronounced effect on weight of the teeth of the low-salt animals than on their calorie controls must be due to the specific lack of mineral salts in the ration.

After realimentation resumption of growth as indicated by body weight occurred (7). A study of the teeth shows that after the shortest period on inadequate mineral supply (3 weeks) realimentation resulted in such acceleration of growth that after 9 weeks the weight of the incisors equalled that of the age controls; but following the longer depletion periods, the subsequent growth was seriously impaired. The incisors both of the low-salt rats and of the calorie controls of the 3 + 9 weeks group weigh more than do those of their age controls. This acceleration might be expected since the growth of the animal as a whole is increased during refeeding to such an extent that the body weights of the low-salt group and of the calorie control group (restricted diet 3 weeks, realimented 9 weeks) exceed the weight of their age controls. This phenomenon of accelerated growth has been described (13) after growth was suppressed by restricted energy intake; according to these investigators, however, the recovery of body weight after salt deficiency was more likely to be incomplete (15). It will be observed that in the cases where the body weight of the experimental rats exceeds that of their age controls (3 + 9 weeks), the weight of the incisors also exceeds that of the controls; whereas in all other groups both the body weight and the weight of the fresh incisors fall short of the respective values for their age control groups. These slight differences, though consistent, are not statistically significant; only in the 12 + 12 weeks group is the mean incisor weight of the realimented low-salt rats

significantly lower than the mean incisor weight of the age controls (S.R.³ = 5.4). This correlation between dental development and somatic growth might be expected inasmuch as Addison and Appleton (1) have observed

TABLE 2
Body weight and weight of the incisors

WEEKS ON EXPERIMENT*	NUMBER OF RATS	MEAN BODY WEIGHT	MEAN WEIGHT FRESH UPPER INCISORS
Low-salt experiment			
3 + 0	Low-salt 10	gm. 129 ± 8.4†	mgm. 136 ± 15.7†
	Age control 7	178 ± 11.8	148 ± 6.4
	Cal. control 4	145	140
6 + 0	Low-salt 11	149 ± 12.7	160 ± 12.8
	Age control 7	236 ± 25.7	191 ± 8.8
	Cal. control 4	184	183
12 + 0	Low-salt 19	155 ± 25.8	192 ± 17.7
	Age control 10	326 ± 41.0	242 ± 21.2
	Cal. control 9	183 ± 9.3	222 ± 14.1
Realimentation experiment			
3 + 9	Low-salt 10	313 ± 56.2	237 ± 26.7
	Age control 8	289 ± 44.5	234 ± 18.2
	Cal. control 4	337	268
6 + 6	Low-salt 11	241 ± 36.5	226 ± 20.6
	Age control 8	289 ± 44.5	234 ± 18.2
6 + 12	Low-salt 10	313 ± 49.8	249 ± 16.3
	Age control 7	358 ± 58.8	281 ± 26.9
	Cal. control 4	334	259
12 + 12	Low-salt 19	307 ± 37.3	249 ± 29.1
	Age control 9	411 ± 28.8	298 ± 18.6
	Cal. control 4	364	275

* Number following plus mark indicates weeks of realimentation.

† Standard deviation.

a close relationship between the size of the head and the mandible and the development of the incisors.

³ S. R. (Significance Ratio) = $\frac{M_1 - M_2}{\sqrt{(SE_1)^2 + (SE_2)^2}}$, where *SE* is the Standard Error
 $\frac{S.D.}{\sqrt{N}}$. When SR is greater than 2 the difference is considered significant.

Relatively slight changes in the chemical composition of the incisors occur during the first three weeks on the low-salt diet (table 3). At 6 weeks, however, the moisture content of the low-salt incisors is significantly higher than that of the age controls (S.R. = 5.0), and the ash content is significantly lower (S.R. = 5.6). The differences are much greater at the end of 12 weeks, for the relative and absolute amounts of moisture in the teeth of the low-salt group continue to increase while in both of the control groups there are decreases. The relative amount of ash, on the other hand, decreases while that of the controls is increasing. It should be emphasized, however, that the absolute amount of ash per pair of teeth of low-salt rats increases about 15 per cent (from 66.3 mgm. to 76.1 mgm.)

TABLE 3
Composition of the incisors in the low-salt experiment

WEEKS ON EXPERIMENT	GROUP	WATER CONTENT		ASH CONTENT	
		Weight, mgm.	Per cent*	Weight, mgm.	Per cent†
3 + 0	Low-salt	47	34.8 ± 3.10	66.3	74.6
	Age control	50	33.6 ± 1.12	73.6	75.0
	Cal. control	46	33.1 ± 1.12	70.0	75.2
6 + 0	Low-salt	62	38.7 ± 3.73	71.1	72.5
	Age control	57	30.3 ± 1.32	101.5	76.1
	Cal. control	54	29.7 ± 1.59	97.7	76.3
12 + 0	Low-salt	80	41.7 ± 4.18	76.1	69.2
	Age control	66	26.9 ± 0.79	133.5	77.1
	Cal. control	60	27.0 ± 1.13	123.9	77.0

* Per cent of fresh weight of the incisors.

† Per cent of dry-extracted weight of the incisors.

during the 12 weeks of the salt-poor regime. The corresponding increases in weight of ash in the age controls is 81 per cent; in the calorie controls, 77 per cent. Thus there occurs a deposition of mineral salts at a greatly reduced rate in the growing incisors, despite the fact that the experimental ration is extremely poor in these essentials. The teeth grow at the expense of bone, for in the latter organ demineralization occurs to a considerable extent (6).

The alterations which occurred in chemical composition of the incisors of the low-salt rats were no longer evident after realimentation (table 4). Statistical treatment of the data on percentages of moisture indicates that the values for the realimented low-salt and age-control groups are in no case significantly different. The largest difference, in the 12 + 12 weeks

group, is 2.3 per cent and is within the limits of the experimental error. In every case the ash of the teeth, based on the moisture- and fat-free organs, had returned to values normal for their age. These findings maintain even though the teeth remain smaller in size than those of their age controls (12 + 12 weeks).

TABLE 4
Composition of the incisors in realimentation experiment

WEEKS ON EXPERIMENT	GROUP	WATER CONTENT		ASH CONTENT	
		Weight, mgm.	Per cent*	Weight, mgm.	Per cent†
3 + 9	Low-salt	64	27.0 \pm 1.69	130.8	76.0
	Age control	63	26.9 \pm 0.69	130.7	76.6
	Cal. control	70	26.6 \pm 1.19	150.0	76.2
6 + 6	Low-salt	61	26.8 \pm 1.78	124.6	75.6
	Age control	63	26.9 \pm 0.69	130.7	76.6
6 + 12	Low-salt	66	26.6 \pm 0.86	138.6	76.4
	Age control	70	24.9 \pm 2.11	161.5	77.0
	Cal. control	66	25.3 \pm 1.04	147.3	76.7
12 + 12	Low-salt	68	27.4 \pm 2.01	137.1	76.1
	Age control	75	25.1 \pm 1.90	170.9	76.6
	Cal. control	73	26.5 \pm 1.10	154.6	77.2

* Per cent of fresh weight of the incisors.

† Per cent of dry-extracted weight of the incisors.

DISCUSSION. The lowered percentages of ash reported here for the incisors of growing rats are of the same order as those reported by Templin and Steenbock (20) for the incisors of adult female rats consuming a ration poor in calcium and vitamin D for about eight months. In their experiments the loss of minerals from the teeth could be prevented by the addition of vitamin D to the diet. Whether the deficient factor be vitamin D or, as in the present experiment, inorganic salts, it appears that the composition of the teeth is much more resistant to change than is that of the bones under similar conditions. Table 5 summarizes the results of five investigators who have analyzed both teeth and bones of rats under a variety of experimental conditions. The incisors cannot be considered a labile source of minerals, particularly when compared to the bones. The present study shows that ash was deposited in the incisors while it was being withdrawn from the bones. Corroborative evidence on this point, supplied by a histological study of the teeth and jaw bone, will appear in a later paper (3).

It has been shown (4, 10, 11) that the pathological condition in the incisal dentin of rats fed rachitogenic diets, can be cured by feeding vitamin D. Similar experiments (23) have shown that vitamin A fed after a

TABLE 5

Extent of change in ash content of incisors and bones of rats, found by various investigators

	ANIMAL USED	REDUCTION IN ASH CONTENT	
		Incisors	Bones
		<i>per cent</i>	<i>per cent</i>
Present study.....	Young ♂ rats	7.9	24.2*
Karshan and Rosebury (11).....	Young rats	6.1	22.7
Templin and Steenbock (19, 20).....	Adult ♀ rats	8.9	10.0
Gies (8).....	Young rats	8.5	20.0
Toverud (21).....	Adult ♀ rats	3.5	13.2

* Details to appear in a later paper.

period when the vitamin was withheld, restored the incisors to normal. These studies have indicated that histological changes which occur in the incisors as a result of dietary measures, are slight compared with parallel changes which occur in the bones, and are readily repaired. The chemical analyses reported herewith support this fact.

SUMMARY

Rats 35 days of age (100 gm.) were fed a diet in which the limiting factor was lack of mineral salts. Marked restriction of somatic growth resulted. The fresh weight of the incisors was affected less than one would expect judging from the general effect on body weight. During the twelve weeks on the low-salt regime the moisture increased relatively and absolutely and the ash decreased relatively in the incisors. The changes in composition reported are completely compensated by refeeding the same diet in which 4 per cent adequate mineral salt mixture has been incorporated, although with the longest salt deficiency period (12 weeks) the fresh weight of the incisors never returned to control values. The ash of the incisors is relatively stable compared to that of the bones: ash is deposited in the growing incisors while it is withdrawn from the bones, under the adverse dietary conditions used in these experiments.

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THE UTILIZATION OF CARBOHYDRATE DURING AEROBIC ACTIVITY IN ISOLATED FROGS' MUSCLE

C. L. GEMMILL

From the Department of Physiology, Johns Hopkins University, School of Medicine, Baltimore, Md.

Received for publication March 23, 1935

The carbohydrate changes in isolated muscle have been studied extensively under anerobic conditions. Meyerhof (1920) found that the glycogen content of ten grams of muscle decreased when the muscle was stimulated sixty times per minute for one-half hour. Groups of muscles stimulated similarly and then allowed to remain in air for twenty-three hours at 14° showed a reformation of carbohydrate and an increased oxygen consumption for that period of time. Many other experiments have been made correlating the lactic acid formation with muscular contraction under anaerobic conditions. From experiments of this type, conclusions have been drawn that the combustion of carbohydrate gave the energy for muscular contraction and that the same cycle of events took place in aerobic as well as anaerobic conditions. For example, Hill and Kupalov (1929) assumed that the combustion of glycogen was the sole source of energy for the sartorius muscle contracting slowly in oxygenated Ringer's solution. From the tension developed, they calculated that this preparation might oxidize as much as one per cent glycogen.

In contrast to the many experiments made on muscles working under anaerobic conditions or stimulated at such a rate that lactic acid is formed even in an atmosphere of oxygen, few attempts have been made to correlate the disappearance of carbohydrate with muscular activity under strictly aerobic conditions. Hill (1928, 1929) has supplied the theoretical and practical data for such conditions. Ochoa (1930), in Meyerhof's laboratory, reported a series of experiments on the oxygen consumption and the carbohydrate content of muscles stimulated at the rate of one stimulus per minute for eighteen to thirty hours. He found that there was sufficient carbohydrate in the normal muscles to account for the calculated disappearance of carbohydrate. However, if the sugar content was reduced by insulin convulsions, the total energy developed was greater than could have been supplied by the complete oxidation of the available carbohydrate in the muscle. The final values for the carbohydrate content of the muscles were not determined but were calculated from the

Km(O₂) ratio obtained in a different series of experiments. Meyerhof (1931) reported similar experiments in which the semi-membranosis was stimulated for nineteen to forty-six hours in oxygenated Ringer's solution. He determined the initial and the final values of the carbohydrate content of these muscles and found that the normal muscles obtained their energy from the oxidation of sugar. However, if muscles had a low sugar content, they might burn other materials for the energy of muscular contraction. In this work, Meyerhof did not report any experiments in which a pair of resting muscles were kept for forty-six hours to see if they had the same content of carbohydrate at the end of that long period of time. Unless that can be proven, it is impossible to take one muscle for the base line and to conclude anything from the variation in content of carbohydrate in the stimulated muscle. Also, Meyerhof did not determine the oxygen consumption of this preparation in order to obtain the total energy exchange.

In view of the lack of data on the total energy used and the carbohydrate disappearance in isolated muscles during aerobic activity, experiments were designed to measure and to compare these two variables in a resting and a stimulated sartorius muscle from the same frog. These experiments would offer proof as to whether the oxidation of carbohydrate was the sole source of energy for muscular contraction in the isolated muscle under aerobic conditions.

METHODS. The method for determining the oxygen consumption was the same as that used by Gemmill (1934) in his study of the respiratory quotient of the frog's sartorius muscle during activity. The total fermentable carbohydrate was obtained by the method described by Cori and Cori (1933).

The first series of experiments was designed to see whether the content of carbohydrate and the utilization of oxygen were the same in two resting sartorii. Three experiments were made in which the sartorii were removed and immediately placed in ice cold sulfuric acid. Another series of determinations was carried out in which the muscles were shaken with the vessels in the water bath for three to six hours before analysis. Following these two series, another group of determinations was made in which one muscle was stimulated with maximal make shocks at the rate of approximately nine per minute for periods of 3.00 to 6.35 hours. The tension was recorded by observing the deflection of a beam of light on a ground glass scale.

RESULTS. The results on the resting muscles are given in table 1. In the three experiments in which the muscles were taken directly for analysis, close agreement was obtained for the fermentable carbohydrate of the corresponding muscles. Greater deviations were observed in the content of carbohydrate in two muscles shaken for periods of three to six hours with the vessels in the water bath, the greatest variation being 0.8 mgm.

per gram of muscle. Therefore deviations of less than this amount would not be significant in determinations of the carbohydrate utilization during stimulation. The average resting oxygen consumption of the muscle attached to the frame was slightly higher than the oxygen consumption of the muscle lying free in the vessel. This was due to the fact that the muscle attached to the frame was under an initial tension which increased the basal oxygen consumption (Feng, 1932). In some of these experiments on resting muscles, two were placed in each vessel since they were very

TABLE 1
Experiments on resting muscles

DATE	NUMBER OF MUSCLES IN EACH VESSEL	DURATION OF EXPERIMENT	OXYGEN CONSUMPTION		TOTAL FERMENTABLE CARBOHYDRATE		CARBOHYDRATE DIFFERENCE	WEIGHT OF MUSCLES	
			I	II	I	II		I	II
1935		hours	mm. ³ per gram/hour	mm. ³ per gram/hour	per cent	per cent	mgm. per gram	grams	grams
Jan.									
10	2				0.42	0.46	0.4	0.102	0.096
11	1				2.86	2.90	0.4	0.139	0.134
12	2				2.16	2.12	0.4	0.176	0.166
15	2	5.0	27.0	29.9	2.58	2.58	0.0	0.147	0.154
16	2	5.66			2.72	2.68	0.4	0.140	0.140
18	1	3.0	39.2	39.4	1.48	1.40	0.8	0.179	0.184
22	1	4.0			1.62	1.67	0.5	0.111	0.104
25	2	3.0	23.0	46.2	2.09	2.16	0.7	0.188	0.168
28	2	5.5	31.3	34.2	1.57	1.60	0.3	0.142	0.130
30	2	6.0	20.2	26.4	1.55	1.63	0.8	0.174	0.157
31	1	6.0	22.6	26.5	2.80	2.81	0.1	0.187	0.168
Averages.....			27.2	33.8					

small. When this was done one muscle from each frog was used in a vessel.

The results obtained on stimulating the muscles are given in table 2. The average oxygen consumption rose from a resting value of 25.5 mm.³ per gram per hour to 606 mm.³ in the stimulated muscle. In a former series, Gemmill (1934) obtained an average value of 275 mm.³ per gram per hour for muscles stimulated seven times a minute. The muscles in the present series contracted at a faster rate; they consumed more oxygen and developed more tension per gram of muscle per hour. However, the ratio between tension developed and oxygen consumed, $Km_{(O_2)}$, was of the same

order of magnitude in the two series. The present determinations gave an average value for the $Km_{(O_2)}$ of 1300 while the former series averaged 1183.

The carbohydrate content of the stimulated muscle decreased in each experiment. Since the amount of carbohydrate in the stimulated muscle was compared with that in a resting muscle which had been shaken for a similar period of time, it was not necessary to subtract any calculated

TABLE 2
Experiments on stimulated muscle

DATE	DURATION OF EXPERIMENT	OXYGEN CONSUMPTION		TOTAL FERMENTABLE CARBOHYDRATE		CARBOHYDRATE DIFFERENCE			TENSION	$Km_{(O_2)}$	WEIGHT OF MUSCLES		LENGTH
		R	W	R	W	Actual	Calculated from O_2	Per cent			R	W	
		mm. ³ per gram/hour	mm. ³ per gram/hour	per cent	per cent	mgm. per gram	mgm. per gram		kgm. per gram/hour		grams	grams	cm.
1935	hours												
Jan. 23	3.17	41.8	668	0.87	0.75	1.2	2.83	42	279	1200	0.129	0.095	4.1
26	6.35	27.8	657	2.12	1.80	3.2	5.67	56	259	1240	0.116	0.110	4.5
Feb. 1	6.25	24.1	566	1.94	1.75	1.9	4.74	40	276	1463	0.113	0.101	4.3
2	6.00	28.8	(238)*	2.19	2.15	0.4	1.92	21			0.140	0.138	
3	6.0	16.9	578	1.35	1.30	0.5	4.64	11	199	1080	0.112	0.108	4.3
4	6.0	27.2	628	2.40	1.96	4.4	5.04	87	252	1235	0.138	0.112	4.4
6	5.0	14.5	652	2.20	1.91	2.9	4.36	66	299	1355	0.104	0.096	4.2
8	6.0	12.8	588	2.57	2.51	0.6	4.73	13	281	1500	0.131	0.121	4.5
Jan. 24	3.0	33.6	(391)*	3.16	2.73	4.3	1.57				0.172	0.180	
29	6.33	27.4	507	1.20	0.67	5.3	4.30		224	1270	0.089	0.079	4.1
Averages...		25.5	606					42	266	1300			

* Not included in general average because tendons broke.

value for the carbohydrate used under basal conditions. Also, if there were any gluconeogenesis, the amount of carbohydrate formed would probably be the same in each muscle. Therefore, changes in carbohydrate content may be attributed to the stimulation. When the actual decrease was compared to the theoretical decrease of carbohydrate as obtained from the oxygen consumption, in only two of the ten experiments was the breakdown of carbohydrate sufficient to account for the total energy exchange. However, these two experiments were not trustworthy. In

the experiment of January 24, the tendon broke at the pelvic end of the muscle, causing damage to the muscle. During the experiment of January 29, the belt slipped off the motor and the apparatus was not shaken for an unknown period of time. The tissue was stimulated during this period and may not have received sufficient oxygen. In the experiment of February 2 the tendon at the tibial attachment broke so the total tension was not recorded in this experiment. Breakage at this end does not damage the muscle as does pulling out of the tendon at the pelvic end. In all of the experiments, except those of January 24 and 29, the actual breakdown of carbohydrate cannot account for the total energy exchange as calculated from the oxygen consumption. The average of the ratio between these two variables was only forty-two per cent. The unfermentable part of the total carbohydrate was small. The average for the resting muscles was 0.18 mgm. per 100 grams of muscle while for the stimulated muscles it averaged 0.27 mgm. This change was so small that it would not affect the general conclusions if it were neglected.

Other factors may account for part of the difference between the actual and the theoretical breakdown of carbohydrate and also for the deviations of this ratio between experiments. Some of these variations were due to experimental error. In the resting series there were deviations of carbohydrate contents between two muscles from 0.0 to 0.8 mgm. per gram of muscle. If the same deviations occurred in the experiments on stimulated muscles they might account for 16 per cent of the variations between experiments.

Another part of these differences might be due to a change in the water content of the stimulated muscles. The wet and dry weights of resting and stimulated sartorii were determined. The water content of thirteen resting muscles shaken in Ringer's solution for seven to nine hours varied from 83.0 to 87.4 per cent. A series of nine experiments in which the muscles were stimulated from 2.7 to 5.5 hours with seven stimuli per minute gave results varying from 83.3 to 86.6 per cent. Since the two series deviated within the same limits, it would be impossible to apply an absolute correction to the results obtained in the present work. These variations, however, would account for some of the differences between experiments.

It has been suggested that the carbohydrate molecule might be partially oxidized without varying its total reducing or fermenting values. Also, carbohydrate might undergo a change in its reducing properties without oxidation. These changes might be intensified by stimulation and produce some of the variations noted in and between experiments. The application of these remote possibilities to the present problem must await the isolation and identification of all the sugars present in resting and working muscles.

The remainder of the deviations between experiments are unaccounted for at the present time. Muscles may oxidize mixed metabolites with variable ratios at different periods during stimulation.

DISCUSSION. Lundsgaard (1930) stimulated muscles poisoned with monoiodoacetic acid in nitrogen and oxygen. He found that more tension was developed and that there was less breakdown of phosphogen when the muscles were contracting in oxygen. Hill and his associates (1931) have reported functional recovery for poisoned muscles in oxygen. This fact suggested that other materials than carbohydrate might supply the energy for aerobic contraction in isolated muscles. The results obtained by Ochoa (1930) and Meyerhof (1931) quoted above indicated also that muscles with low carbohydrate content may derive part of their energy for contraction from non-carbohydrate sources.

Gemmill (1934) reported that the respiratory quotient of an isolated muscle rose on stimulation from a resting value of 0.80 to 0.90. He concluded that this rise indicated that the energy for contraction in a "steady state" was not obtained exclusively from the oxidation of carbohydrate. The direct chemical comparison of the total energy expended for contraction and the utilization of carbohydrate in muscles contracting under aerobic conditions has shown this to be the case. The energy for contraction in an isolated muscle comes only in part from the oxidation of carbohydrate.

In Meyerhof's laboratory, Gemmill (1932) showed a direct proportionality between the lactic acid produced and the tension developed in a sartorius muscle stimulated slowly in oxygen-free Ringer's solution. This proportionality was found to hold for values of lactic acid as high as 1.2 per cent. In the present series, no relationship was found between the aerobic breakdown of carbohydrate and tension developed by the muscle. Therefore, it is very probable that chemical events occurring in aerobic contractions may be entirely different from those taking place under anaerobic conditions. It could be assumed, however, that a mixed metabolism was used to reconvert lactic acid into glycogen. Experiments of the present type, however, tell nothing concerning the chain of chemical processes taking place before the final oxidations. Much more work will be needed before the chemical changes occurring during aerobic and anaerobic activity can be coupled into a satisfactory explanation of muscular contraction.

SUMMARY

A comparison was made of the oxygen consumption and the utilization of total fermentable carbohydrate during activity of frogs' isolated sartorii. The muscles were stimulated nine times a minute for periods of three to six hours in oxygenated Ringer's solution. The average utilization of carbohydrate accounted for only 42 per cent of the total energy

exchange as obtained from the oxygen consumption. Therefore other material than carbohydrate is oxidized to supply the energy for contraction of isolated frogs' muscle under aerobic conditions.

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EVIDENCES OF AN ALTERED TISSUE STATE IN VENTRICULAR FIBRILLATION

N. D. KEHAR AND D. R. HOOKER

From the Department of Physiological Hygiene of the Johns Hopkins University

Received for publication February 21, 1935

The present paper makes three specific contributions pointing to an altered tissue state of physico-chemical nature in ventricular fibrillation; *a*, lowering of the freezing point; *b*, liberation of potassium, and *c*, modification of microscopic structure. All the results reported are based upon observations of the isolated dog heart perfused with Locke solution. Fibrillation was induced by tetanic stimulation of the ventricles and recovery from fibrillation with return of a normal sequential rhythm was obtained by the injection of about 2 cc. of 3 per cent KCl into the perfusion cannula.

Freezing point. Pieces of ventricular tissue of approximately equal size, ± 10 grams in weight, were cut from the same heart, 1, after perfusion for 15 minutes; 2, after 5 minutes of fibrillation during which perfusion was continued, and 3, after a second period of perfusion for 15 minutes subsequent to a recovered normal beat obtained by the use of strong KCl. The pieces were blotted dry and placed in small vials with rubber caps which were submerged in an ice-salt mixture.

The freezing point was determined by the thermocouple method devised by Karrer and Estabrook (1) to whom we are indebted for assistance in its application. The thermo-junction of iron and constantan in a no. 24 hypodermic needle was thrust through the rubber cap and buried near the center of the tissue mass. The other similar junction of heavier wire was kept at 0°C. in a Dewar flask, the two junctions being connected through a galvanometer which registered a deflection of 6.5 cm. for each 1°C. This method has particular merit because slight injury is done to the structure of the tissue. In this manner cooling curves were obtained for each piece of tissue as shown in figure 1. Six such experiments were performed, all in substantial agreement.

The curves show common characteristics, a brief super-cooling which could usually be stopped by slight jarring of the vial, a rise to a plateau and a subsequent fall. When the super-cooling is disturbed solidification of the tissue begins and the heat given off thereby stabilizes the temperature and produces the plateau which is taken as the freezing point. As

the curves shown in figure 1 indicate the freezing point of the fibrillated tissue is appreciably below that of the control and recovered specimens, the latter two tending to be of the same value. This result means that in the state of fibrillation the inorganic constituents of the tissue are in a relatively disaggregated condition.

Liberation of potassium. In a search for correlating evidence to accord with the fore-going observations analyses were made of the out-flowing perfusate before and after the establishment of ventricular fibrillation. These analyses concerned two groups of inorganic substances. First those known to be normal constituents of animal tissue but not present in the perfusing solution (S, P, Mg, Mn, I, Al, Co, Cu, N and Zn); these were negative without exception. Second those present both in the tissue and in the perfusate (Na, Ca and K); in this case comparison had to be made

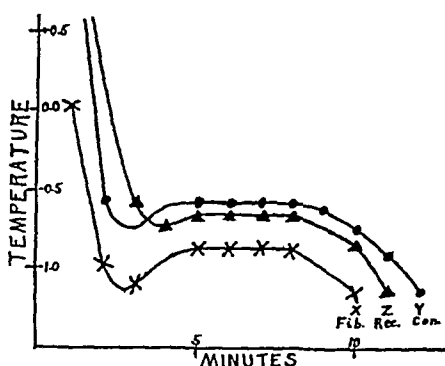


Fig. 1. Cooling curves showing freezing point (plateau) of ventricular muscle.

between the outflowing perfusate before and after the onset of fibrillation. The results for sodium and calcium were likewise negative.

In the case of potassium, however, the results were positive. While the potassium content of the perfusate was unchanged by passage through the normally beating heart, immediately after fibrillation began the outflowing perfusate showed an appreciable increase in the amount of potassium present. The analytical method of Kerr was followed (2). Although it is realized that potassium analyses are beset with pitfalls we believe our methods to be trustworthy since every safeguard against error was taken and more especially since the samples were given blind numbers so that the analyses were made without prejudice. In spite of the uncertainties inherent in the method used this finding is important because of the influence exerted by potassium on the normally beating heart and because potassium may be used to recover normal behavior in a heart thrown into ventricular fibrillation (3).

In nine experiments the average potassium content of the out-flowing

perfusate, in milligrams per cent, before fibrillation was 12.8 and shortly after fibrillation it was 15.0. These values are relatively comparable. The actual amount of potassium in the perfusate varied somewhat in different experiments and the time of collection of the second sample also varied in different experiments. The results, however, were concordant in all experiments and point to a primary upset in the tissue metabolism which is somehow related to the liberation and outward diffusion of potassium ions.

This liberation of potassium would thus appear to be in direct relation to the onset of fibrillation. Samples collected one-half to one minute after fibrillation was established showed the maximum amount of potassium while those taken after fibrillation had continued 3 to 4 minutes often showed a return to the pre-fibrillation value.

This association of readily diffusible potassium with the onset of ventricular fibrillation produced by mild electrical stimulation, however attractive as a working hypothesis, must be definitely qualified. It has been shown that the application of severe electrical stimulation to the heart not only fails to establish ventricular fibrillation but is actually effective in restoring a fibrillating heart to normal functional behavior (4).

In the course of these experiments opportunity was had in two cases to analyse the potassium content of the outflowing perfusate before and after the application of an electrical stimulus of a strength above that which will cause fibrillation. The average values in milligrams per cent were 12.7 and 14.1 respectively. The difference here is less (1.9) than when a fibrillation inducing stimulus was employed (2.2) but because of the limitations of the analytical method and because only two observations were made little emphasis can be placed on the point. Thus with the evidence at hand we can not say that there is a specific relationship between the liberation of potassium by the muscle fibre and the onset of fibrillation.

Modification of microscopic structure. The heart of an anesthetized intact dog is readily thrown into ventricular fibrillation by the passage of a suitable electrical current with resultant death. Post-mortem histological examination of the cardiac muscle under such conditions shows no abnormality. Similarly frog sartorius muscle thrown into a twitching state by passage from Ringer solution to sodium chloride solution is perfectly normal in histological appearance. These points are to be held in mind in connection with the following presentation.

When the perfused and isolated dog heart is thrown into ventricular fibrillation and bits of tissue are examined microscopically the histological picture is that shown in figure 2. Our procedure here was similar to that in obtaining tissue for freezing point determinations, i.e., bits of ventricular muscle were cut off before, during and after fibrillation. They were at once dropped into Zenker's fluid and subsequently examined by Dr. A. R.

Rich to whom we are greatly indebted for this assistance and for the following report:

The fibres of the myocardium of the heart in fibrillation are altered in an extraordinary way which can be best appreciated by an examination of the microphotographs. Briefly, the lesion consists in hyalinization and swelling of the ends of the individual fibres. In these swollen, homogeneously pink-staining areas no striations are visible, but the striations in the remainder of the same fibre are plainly seen, and it is perfectly clear that only a part of the fibre is involved in the hyalinizing process. The nuclei appear quite normal. This curious alteration of the fibres occurs in scattered foci, the muscle fibres between the foci remaining quite normal.

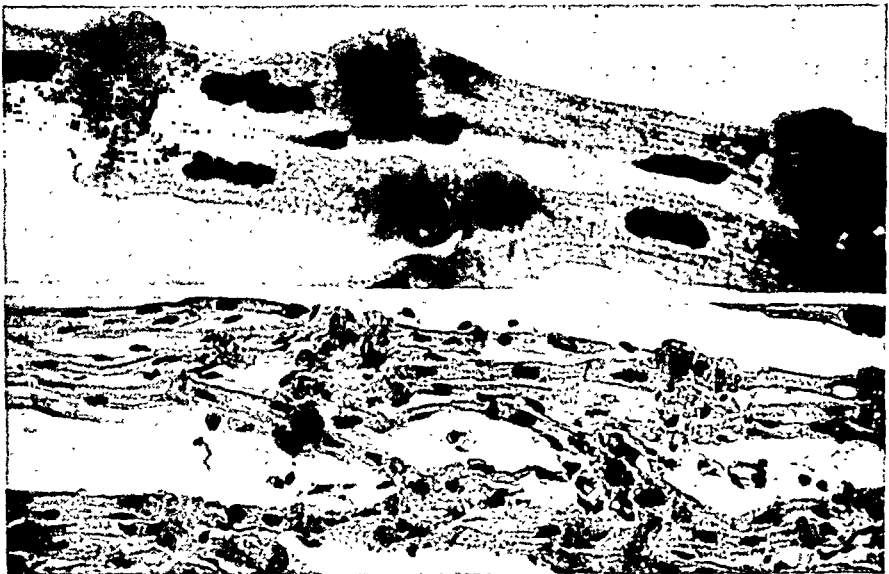


Fig. 2. Shows high and low magnification of ventricular muscle taken from perfused heart in fibrillation.

One is at once reminded of the familiar hyaline degeneration of skeletal muscle which occurs so commonly in the human being, for the appearance of these heart muscle fibres is precisely like that in the hyalinized skeletal muscle fibres. Hyalinization of skeletal muscle is ordinarily regarded as representing an irreversible change in a dead fibre, but if one examines such lesions carefully it is not unusual to see that only a part of an individual fibre is hyalinized while the striations of the rest of the fibre are quite normal. In view of the fact that the present material indicates that rapid recovery from the hyalinized state is possible when it affects only part of the fibre, one is disposed to wonder whether the partial hyalinization of a skeletal muscle fibre may not also represent only a temporary alteration. When one looks at these hyalinized areas in the heart muscle it seems incredible that they could rapidly disappear, with the return of the striations to the area, and perhaps the limitations of the experiment, which prevent one from examining areas from the entire heart during fibrillation and after recovery, may be responsible for the fact that the areas

taken during fibrillation show much more extensive foci of hyalinization than do those taken before fibrillation or after recovery. The only way in which one might obtain more definite information about the possibility of the disappearance of the change on recovery from fibrillation would be to study a much larger series of hearts under the same conditions.

I have never seen a change quite like this in human heart muscle, whether in persons dying with auricular or ventricular fibrillation, nor have I ever seen it in the hearts of animals at autopsy. Hyalinization of dead fibres does, indeed, occur in the human being in various pathological conditions, and focal hyalinization of the type described by German writers as "koernig-scholliger Zerfall" is also occasionally observed in infections; but the present lesion, localized as it is at the ends of the individual fibres, is quite unlike anything that I have ever seen.

It is of interest that the change was completely absent when the dog's heart was thrown into fibrillation within the animal's body and, on the other hand, that an occasional hyalinized fibre was found in the hearts perfused outside the body even before general fibrillation had been induced. This indicates very strongly that the occurrence of the lesion is dependent in part at least upon the abnormal conditions attendant upon the process of artificial perfusion, and this perhaps should place one on guard against applying too readily to the intact heart results which are obtained during perfusion outside the body, for such a profound morphological alteration as this is must surely be associated with definite chemical and metabolic alterations.

The points of immediate interest in this report are: *a*, the exaggeration of the condition in fibrillation, and *b*, the reversible nature of the condition. Thus while we are dealing with a tissue which is not wholly normal since some degree of hyalinization is present in control specimens (a condition not present in hearts similarly fibrillated but with the circulation intact) it exhibits the remarkable capacity to revert to its own normal when the causative disturbance has been counteracted.

DISCUSSION. The foregoing evidence while not conclusive at all points has cumulative force. We have shown, subject to the reservations stated, that the perfused ventricular muscle in passing into the state of fibrillation undergoes a change such that the freezing point of the tissue is lowered. This change is accompanied by a visible alteration in the microscopic structure of the muscle fibre. We have further shown that these changes in molecular aggregation and anatomical configuration are reversible processes. Along with these are the additional facts that of all the inorganic constituents present in the muscle potassium alone undergoes a change by outward diffusion and that momentary increase in the potassium content of the perfusate brings about a complete recovery from fibrillation. Circumstantially, therefore, potassium seems the most probable incriminating factor in relation to ventricular fibrillation.

CONCLUSIONS

When the perfused dog heart is thrown into ventricular fibrillation by electrical stimulation the following changes occur: *a*, the freezing point

of the tissue is lowered; *b*, the microscopic picture of the tissue is changed, and *c*, the potassium content of the out-flowing perfusate is increased. The first two of these changes are reversed by a momentary increase in the potassium content of the perfusate which at the same time has the effect of restoring a normal sequential beat in the heart.

It is therefrom postulated that an unbalance of the readily diffusible potassium ion is associated with the phenomenon of ventricular fibrillation.

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WEIGHT LOSS CHANGES DURING MUSCULAR WORK FOLLOWING FOOD INGESTION

CARL IVER HOVLAND

Institute of Human Relations, Yale University¹

Received for publication April 3, 1935

It has frequently been suggested that efficiency of performance can be measured quantitatively by using as a criterion the amount of energy consumed in doing a given amount of work. For this purpose oxygen consumption has often been employed as an index of the amount of energy consumption. Numerous studies on the effect of muscular work upon energy transformations have shown marked increases in oxygen consumption, minute volume of the heart, pulse rate and the like. The work of Lovekin (2) shows how these physiological indices may be used in the determination of the efficiency of work. But the difficulties involved in using metabolic indices for this purpose are immediately apparent. There are numerous other factors which affect the metabolism of the organism, and there may be considerable changes taking place due to food, temperature, etc., which have no relevance to efficiency. The plan of the present research is to calculate the ratio between metabolism during work and that during relaxation for determining the changes taking place in the energy used up by the organism, and to use this index rather than the absolute values of metabolism. In this way changes which are brought about by extraneous factors will not affect our results. We are using as our metabolic index the amount of weight lost per unit of time. Our principal interest in the present study is the relative amount of weight lost in doing an equivalent amount of work at various hours following the ingestion of large and small meals.

METHOD. For the purpose of obtaining accurate readings of weight loss in short intervals of time the Sauter balance was used. The cot upon which the subject rested was mounted directly upon one beam of the balance and counterbalanced on the other side by weights. Sufficient weight was placed upon the scales so that the subject was only slightly heavier than the counterbalance. At the exact moment when the subject and the weights came into equilibrium a stop watch was started. Two additional grams were placed upon the subject's side by the experimenter

¹ Done in the Department of Psychology, Northwestern University. The writer expresses deep appreciation to Prof. G. L. Freeman for facilities and assistance.

and the time required for the subject to lose these two grams was obtained by stopping the watch when equilibrium was again reached. Three such determinations were made while the subject was in a relaxed condition. Three more readings were taken while muscular work was being performed. The task consisted of flexing and relaxing the index finger of the left hand as rapidly as possible. A work adder was mounted on the cot so that the amount of work done was recorded without requiring the subject to change his body position.

Ten normal, healthy subjects were employed. Their age range was from 18 to 34 years, their weight from 132 to 178 pounds, and their height

TABLE 1

Weight loss during relaxation and during finger oscillation following the ingestion of a light lunch

	BEFORE IN- GESTION	HOURS AFTER FOOD				
		1	2	3	4	5
Loss per hour during relaxation.....	33.8	36.5	37.1	35.4	34.4	34.2
Loss per hour during work.....	35.2	39.1	40.9	38.6	41.3	40.4
Ratio of loss during work to loss during relaxation.....	104	107	110	109	120	118

TABLE 2

Weight loss during relaxation and during finger oscillation following the ingestion of a heavy lunch

	BEFORE IN- GESTION	HOURS AFTER FOOD				
		1	2	3	4	5
Loss per hour during relaxation.....	34.5	38.5	40.0	42.4	38.5	36.1
Loss per hour during work.....	37.3	47.0	45.6	49.1	48.1	43.7
Ratio (in per cent) of loss during work to loss during relaxation.....	108	122	114	116	125	121

from 64 to 71 inches. At the commencement of the experimental day the subjects were in a basal, post-absorptive condition.

The meals were given at twelve o'clock noon. Weight loss readings were taken hourly, the first one being just before food ingestion. The subjects continued to relax on their cot throughout the experiment. Room temperature was maintained at 70 degrees Fahrenheit. Light, loose clothing was worn by the subjects.

After a day of preliminary orientation to the apparatus and procedure, the subjects were tested for four days. On the first and third day they

were given a light meal, described below, and on the second and fourth day a heavy meal. Half of the subjects were given the light meal first, and half were given the heavy. The light meal consisted of one ham sandwich and one glass of milk; the heavy meal was prepared by tripling the above rations.

EXPERIMENTAL RESULTS. Table 1 gives the number of grams lost per hour in the relaxed condition, the number lost during the finger oscillation and the ratio of loss during oscillation to loss during relaxation (in per cent) following the ingestion of the light meal. In table 2 are given the same data for the ten subjects after the ingestion of the heavy meal.

DISCUSSION. It will be observed that the changes in weight loss following the intake of large and small meals during relaxation are identical with those shown in an earlier paper of the writer (1). There is a greater rise following the heavy meal and its peak is later than the rise following the light meal.

The ratios of weight loss during the finger oscillation to the loss during relaxation show that in terms of energy expenditure a light lunch is more favorable to efficient performance than is a heavy meal. Its advantage is most marked in the earlier part of the afternoon. There is a general rise in energy cost, with the maximum increase in weight loss during work over that required during relaxation at four o'clock.

The changes correlate very closely with subjective impressions of the efficiency of performance which were obtained from the subjects. Their statements indicated that they had a feeling of sluggishness following the heavy meal, a fact which is reflected in the greater weight loss required to perform a standard amount of work, and that there was a late afternoon slump, also reflected in greater expenditure at four o'clock. The end spurt at the conclusion of the work is marked, correlating with relief at the end of the day's work.

SUMMARY

Weight loss was recorded during relaxation and during the performance of finger oscillation following the ingestion of large and small meals. Ten subjects were used four days each. Efficiency, as judged by the increment of weight loss during work as compared to the loss during relaxation, appears better after a light lunch, the advantage being greatest in the earlier part of the afternoon, immediately after eating. Maximum inefficiency, from the standpoint of energy expenditure, is found in the late afternoon, about four hours after eating.

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THE RÔLE OF THE HYPOPHYSIS IN EXPERIMENTAL CHRONIC ADRENAL INSUFFICIENCY¹

ARTHUR GROLLMAN² AND W. M. FIROR³

From the Department of Pharmacology and Experimental Therapeutics, and the Department of Surgery, The Johns Hopkins University

Received for publication April 2, 1935

It is generally assumed that the adrenal glands and the hypophysis are mutually related in their physiological functions, for extirpation of either of these organs is accompanied by anatomical changes in the other.

Clinically, one observes changes in the pituitary in diseases involving the adrenal cortex and changes in the adrenal in acromegaly, hypophyseal cachexia and other pathological involvements of the hypophysis.

We have observed that animals maintained for some time in a state of chronic adrenal insufficiency assume a clinical picture which is essentially that of hypophyseal cachexia. The physiological deficiencies manifested by such animals are not remedied by treatment with the adrenal cortical hormone but respond to treatment with extracts derived from the pituitary. We must conclude, therefore, that the hypophyseal insufficiency induced by interference with normal adrenal activity is primarily responsible for producing an essential part of the syndrome observed in animals suffering from chronic adrenal insufficiency.

Experimental chronic adrenal insufficiency. Several methods of inducing chronic adrenal insufficiency have been utilized in the present study. The first consisted in treating completely adrenalectomized animals (rats, cats and dogs) for extended periods with a minimal amount of the adrenal cortical hormone sufficient to maintain life but in insufficient dosage to maintain growth in young animals or to maintain normal body temperature, body metabolism, activity of the reproductive organs and general normal activities in adult animals. It is extremely difficult to maintain such a state of insufficiency in young animals for an extended period of time but in matured full-grown animals it may be done with ease.

The second method of inducing a chronic adrenal insufficiency consisted

¹ Studies on the adrenal. IXth communication.

² Aided by grants from the Ella Sachs Plotz Fund and the National Research Council for which we wish to express our appreciation.

³ Aided by grants from Mr. Stephen C. Clark and the Hartley Corporation for which we are greatly indebted.

in an incomplete adrenalectomy of one-month-old rats. The operation being incomplete, cellular residues remained which eventually gave rise to sufficient gland to maintain life after treatment of these animals for a week to ten days (Grollman and Firor, 1933) with an active preparation of the adrenal cortical hormone.

A third procedure for producing a chronic insufficiency consisted in ligating the blood supply to the adrenals. Although a large percentage of the animals on which this operation was performed succumbed to the effects of an ensuing acute adrenal insufficiency, some survived and developed the symptoms of chronic insufficiency.

The general symptomatology of animals in a state of chronic adrenal insufficiency induced by the methods just described differs from that observed in acute adrenal insufficiency in several important respects. Acute adrenal insufficiency responds readily to treatment with the adrenal cortical hormone, is rapidly fatal in untreated animals, and is accompanied by a rapid loss of weight and other symptoms characteristic of adrenal insufficiency. Animals brought into the state of what we shall describe as a chronic adrenal insufficiency live for long periods of time without treatment, do not respond to adrenal cortical therapy, maintain a constant body weight and are free of many of the symptoms characteristic of acute adrenal insufficiency. Such animals show a slight degree of asthenia, fail to gain weight under a *luxus* diet, maintain a slightly lowered body temperature, and show a diminished capacity for reproduction. At autopsy evidences of pathological changes are noted chiefly in the degree of inanition and loss of general body fat, marked atrophy of the reproductive system, atrophy of the thyroid gland, and a generalized hyperplasia of the lymphatic system with regenerative enlargement of the thymus. This hyperplasia of the thymus occurs in adult cats and dogs as well as in rabbits and rats, in which it has been frequently observed by previous workers.

The time necessary for inducing this condition of chronic adrenal insufficiency depends chiefly upon the age of the animal and the degree of adrenal insufficiency to which the animal is subjected. In adult rats, cats, and dogs, the condition which we shall describe in the subsequent sections requires several months for its development. Our observations were carried out during the third month following bilateral adrenalectomy, the animals being maintained from the time of operation on an amount of adrenal cortical hormone sufficient to maintain life but insufficient to maintain normal physiological activity. In month-old rats, on the other hand, an acute insufficiency induced by the second method described in the preceding section will give rise to the condition after a period of only a few weeks. In such animals, also, insufficient therapy for as short a period as a week will often induce an irresponsiveness to subsequent adequate ther-

apy which, as we shall see, is due to the development of secondary changes which in turn are responsible for many of the manifestations of chronic adrenal insufficiency.

Growth of rats in chronic adrenal insufficiency. The growth of rats may be stunted by interference with normal hypophyseal or adrenal function. In figure 1 have been reproduced the growth curves of rats, illustrative of

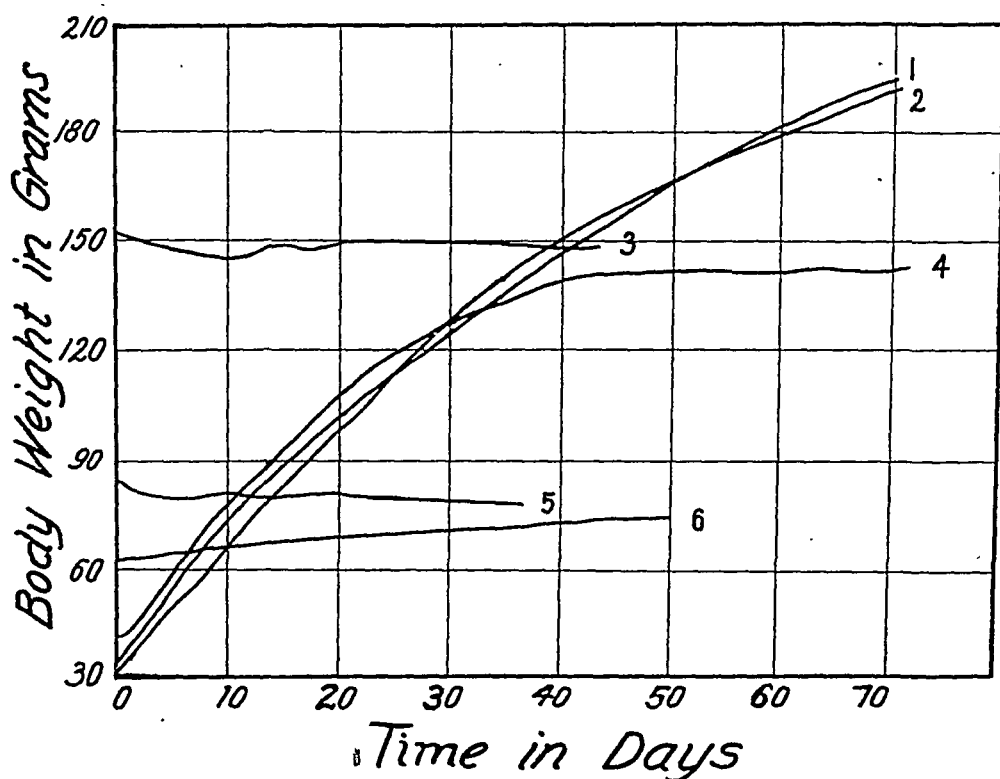


Fig. 1. The growth curves of female rats under the following experimental conditions:

Curve 1, normal unoperated control; 2, adrenalectomized and adequately treated with the adrenal cortical hormone; 3, adrenal pedicles ligated; 4, incompletely adrenalectomized; 5, hypophysectomized; 6, completely adrenalectomized but treated with an inadequate dose of the adrenal cortical hormone. All operations were performed at the time indicated as 0 on the abscissae.

typical reactions to various types of adrenal or hypophyseal dysfunction. Adrenalectomy results in a permanent cessation of growth which can be entirely prevented by adequate replacement therapy with the adrenal cortical hormone (Grollman, Firor and Grollman, 1935). Curve 2 of figure 1 shows the normal growth of such a treated rat as compared with the unoperated litter-mate control illustrated in curve 1.

Failure to treat an adrenalectomized rat results in death in the course

of a few days to a month depending upon the age and condition of the animal (Jaffe; Pencharz, et alii; Firor and Grollman). However, should the adrenal cortical hormone be administered in small doses one is able to prolong the life of adrenalectomized young animals without supplying sufficient hormone to permit growth. Stunting of growth in this manner is illustrated in curve 6 of figure 1. Incomplete removal of the adrenals, leaving only a minute portion of the glomerulosa of the gland, suffices for regeneration of the gland (Pencharz, Olmsted and Giragossintz) and prolonged survival. In many cases such animals assume their normal adult size and manifest no symptoms of adrenal insufficiency. In some cases, however, one observes that such animals after a preliminary period of growth ultimately cease to grow and maintain a constant weight for long periods of time as illustrated in curve 4 of figure 1. The preliminary normal growth observed in this animal is similar to that noted by Collip, Selye and Thomson in hypophysectomized young rats in which growth does not cease until some time after operation.

Curve 3 illustrates the stunting of growth observed in an animal in which the adrenal pedicles were ligated. Cessation of growth in this animal is similar to that noted in curve 5 which illustrates the cessation of growth in a rat after hypophysectomy.

Effects of adrenal and hypophyseal therapy on growth in rats. In figure 2 are reproduced typical curves which show the effects of administering the adrenal cortical hormone or the growth hormone of the anterior pituitary body to rats whose growth had been stunted by the various procedures cited in the preceding section. The periods of treatment extended for 20 successive days, each period of treatment alternating with periods of an equal length of time during which no treatment was administered. Many animals succumbed before the expiration of the long experimental period cited for the animals in figure 2. However, each part of the experiment, i.e., a 60-day experiment in which the animals were maintained untreated for 20 days, received either adrenal cortical or anterior pituitary growth hormone for 20 days, and finally were observed for 20 more days without treatment, was repeated six times on groups of three rats with the same results as are shown in the more prolonged experiments of figure 2.

Adrenal cortical hormone was administered either in the form of an extract (Grollman and Firor, 1933) injected intraperitoneally daily or in the more satisfactory form of the charcoal-hormone preparation administered orally (Grollman, Firor and Grollman, 1935). To ensure adequate therapy at least 3 rat units of hormone (Grollman and Firor, 1934) were administered daily. The growth hormone of the anterior pituitary was administered intraperitoneally in 1 cc. doses. Part of the extracts were prepared by the method of Putnam, Teel and Benedict as modified by Bugbee, Simmond and Grimes. A satisfactory preparation was also gener-

ously supplied by Dr. J. A. Morrell of E. R. Squibb and Sons, to whom we wish to express our indebtedness.

As shown in figure 2, administration of the adrenal cortical hormone is

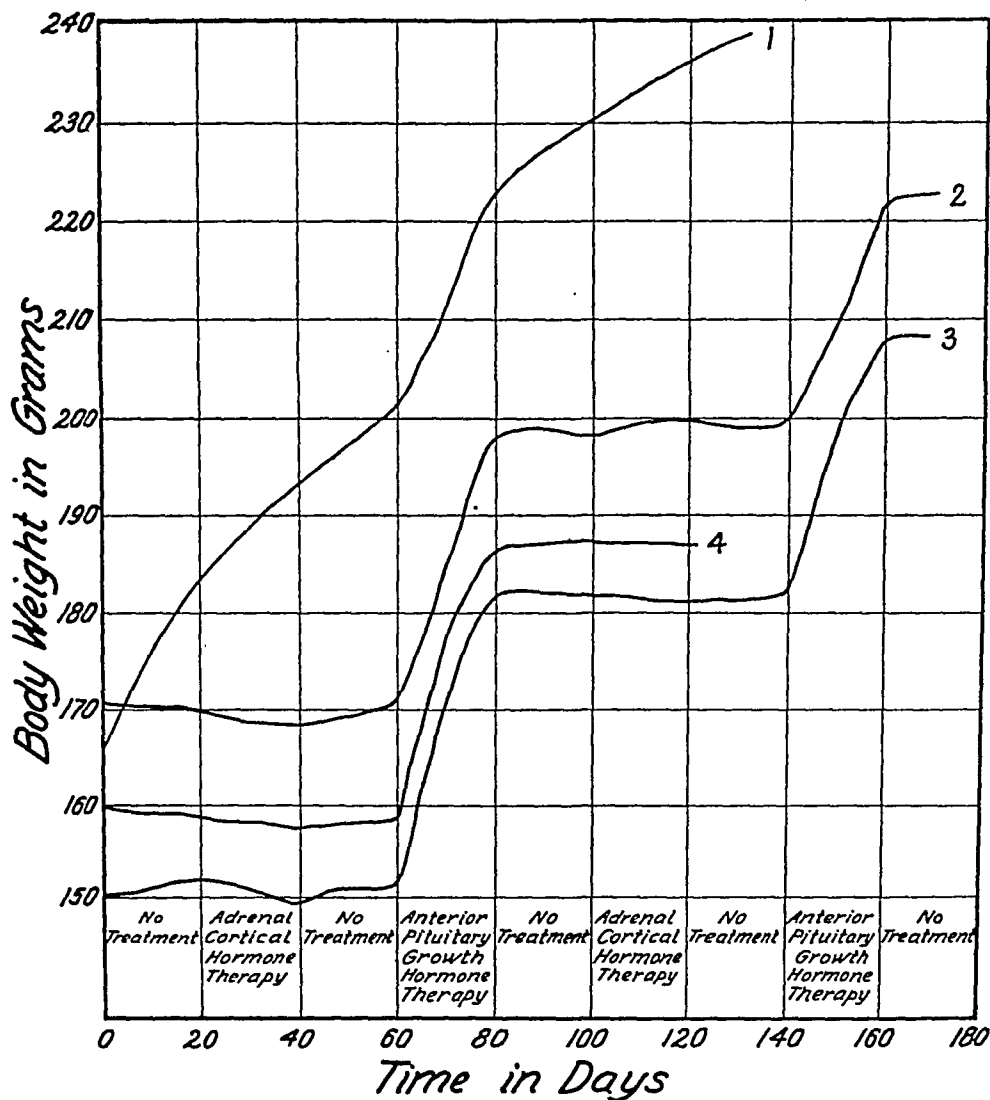


Fig. 2. The effect of adrenal cortical and anterior pituitary growth hormone therapy on the growth-curves of female rats under the following experimental procedures:

Curve 1, normal unoperated control; 2, adrenal pedicles ligated; 3, incompletely adrenalectomized; 4, hypophysectomized.

The animals were given no treatment during the twenty day periods indicated on the abscissae as 0 to 20, 40 to 60, 80 to 100, 120 to 140 and 160 to 180 days. Each animal received 3 rat units of the adrenal cortical hormone daily during the periods of 20 to 40, and 100 to 120 days. They received 1 cc. of anterior pituitary growth hormone extract daily during the periods of 60 to 80 and 140 to 160 days.

without the least effect on the growth of rats stunted by chronic adrenal insufficiency or by hypophysectomy. On the other hand the administration of extracts of the anterior lobe of the pituitary is accompanied by a remarkable growth approximating or exceeding that observed in normal animals. It may be concluded, therefore, that the cessation of growth in animals maintained for long periods in chronic adrenal insufficiency is not due to lack of the adrenal secretion but is due to pituitary insufficiency induced secondarily by the adrenal insufficiency.

The reproductive system in chronic adrenal insufficiency. The reproductive function of animals in adrenal insufficiency is in abeyance as numerous observers have demonstrated. We have verified this on a large series of rats, cats and dogs maintained for long periods in a state of chronic insufficiency. In the rat, in which, because of the shortness of the oestral cycle, these observations are best followed, the periods of diestrus may extend for months during chronic adrenal insufficiency. However, in animals in which growth has ceased for several months, spontaneous oestrus may still occur at intervals of several months, compared to 4 to 7 days for normal rats. The animals conceive and may rear a normal litter. About half the animals die during pregnancy or lactation. In such cases there is no evidence of failure of mammary function and the young are normally nourished. Undoubtedly, the strain of pregnancy like other strains upon the organism (excessive heat, cold, drugs such as histamine, etc.) which are innocuous to normal animals can not be borne by animals in chronic insufficiency. In animals surviving the period of lactation, the body weight is found to be the same as before pregnancy. Any growth hormone in the fetuses is apparently not transmitted to the mother, for the stunting of growth continues throughout life. A second pregnancy occurs rarely.

Male animals in chronic adrenal insufficiency show impotence with atrophy of the reproductive system similar to that observed in hypophysectomized animals.

In order to determine whether the adrenal or the hypophysis is responsible for the dysfunction of the reproductive system, hormones derived from these glands were administered to female rats for several months. Repair of the reproductive system did not occur when adrenal cortical hormone was administered in doses of two to three rat units daily. On the other hand, a ready response, similar to that obtained in hypophysectomized animals (Smith), was elicited by injections of extracts of the pituitary, prepared by the method of Fevold, Hisaw and Leonard.

The above observations indicate that the hypophysis is probably responsible for the observed failure of the reproductive system in long continued chronic adrenal insufficiency. The fact that normal reproductive activity can occur in animals whose growth has been permanently stunted

is physiological evidence for the separateness of the growth and reproductive functions of the hypophysis.

The body temperature in chronic adrenal insufficiency. Certain metabolic changes are common to both hypophysectomized animals and those maintained in a state of chronic adrenal insufficiency. Thus, the body temperature is reduced about equally in both cases. In neither hypophysectomized animals nor those maintained in a state of chronic adrenal insufficiency for long periods could the body temperature be elevated to normal by administration of the adrenal cortical hormone. On the other hand, administration of either desiccated thyroid, orally, or the injection of thyroxine⁴ or anterior pituitary extracts containing the thyrotropic principal resulted in an elevation of the body temperature to normal. In

TABLE 1

The body temperature of animals in a state of chronic adrenal insufficiency

The data in the table represent averages of daily temperatures obtained during the course of one week. The average deviation of the individual readings from the mean values recorded in the table was less than 0.3°.

NUMBER OF ANIMALS IN SERIES	ANIMAL SPECIES	OPERATIVE PROCEDURE	RECTAL TEMPERATURES		
			Untreated	During thyrotropic hormone therapy	During thyroid medication
			°C.	°C.	°C.
4	Dogs	Normal controls	39.6		
2	Dogs	Hypophysectomized	38.7	39.8	39.5
2	Dogs	Chronic adrenal insufficiency	38.6	39.7	39.8
8	Rats	Normal controls	39.1		
4	Rats	Hypophysectomized	36.5	39.2	38.7
4	Rats	Chronic adrenal insufficiency	36.6	39.3	39.0

table 1 are summarized the results obtained on a series of rats and dogs hypophysectomized or in chronic adrenal insufficiency. The results of table 1 may be interpreted as due to successive changes in the *adrenal-pituitary-thyroid* complex. The primary adrenal insufficiency causes an irreversible injury of the anterior pituitary. This pituitary dysfunction, in turn, results in a thyroid insufficiency which gives rise to the observed symptoms.

The above interpretation of the thyroid deficiency in chronic adrenal insufficiency avoids the paradox otherwise presented by the observations of thyroid hyperactivity in acute adrenal insufficiency (Marine). The latter condition may be considered as a direct effect of adrenal insufficiency

⁴ We are indebted to E. R. Squibb and Sons for a generous supply of crystalline thyroxine utilized in this work.

while the results which we have described for chronic insufficiency are due to a secondary pituitary dysfunction.

Anatomical considerations. Histological examination of the anterior lobe of the hypophysis of animals subjected for long periods to adrenal insufficiency revealed changes which may be taken as indicating the anatomical basis for the physiological deficiencies described in the preceding sections.

Dogs maintained on minimal doses of adrenal cortical hormone for periods of 100 days and then allowed to die of adrenal insufficiency revealed changes in the pituitary which resemble those reported in patients dying of Addison's disease (Kraus, 1927). There was an increase in vascularity of the hypophysis, dilatation of the capillaries, and a marked diminution in the number of basophilic cells which, in one dog, had completely disappeared. In the rat, the increased vascularity was less striking than in the dog and there was not as marked a diminution of the basophilic cells. However, the staining of these basophilic cells was very abnormal; As in Addison's disease the changes in the eosinophilic and chromophobic cells were not as striking as the changes observed in the basophilic cells.

Histological examination of the reproductive organs and the thyroids of animals maintained for long periods in chronic adrenal insufficiency demonstrated the same atrophic condition as has been described for hypophysectomized animals (Evans, Smith).

Discussion. It should be emphasized that the observations cited in the present paper were obtained only in animals maintained for a long period in chronic adrenal insufficiency. It apparently requires a prolonged period of mild adrenal insufficiency or a shorter period of an acute insufficiency to induce an irreparable injury to the hypophysis. It is quite possible that other symptoms of chronic adrenal insufficiency than those described here (e.g., the enlargement of the thymus, disturbances in carbohydrate and fat metabolism, etc.) may also be due to secondary hypophyseal dysfunction rather than to the primary adrenal injury. It is significant to note in this connection that the chronic adrenal insufficiency induced by an incomplete adrenalectomy in young rats occurs despite the presence of considerable regenerated adrenal cortical tissue. Since this regeneration occurs rapidly it is logical to suspect extra-adrenal factors as being responsible for the ultimate manifestations which we have described.

There have been attempts recently to attribute the failure of patients suffering from Addison's disease to respond to extracts of the adrenal cortex to involvement of other organs than the adrenals. Although secondary pituitary insufficiency may be expected to occur in Addison's disease (as is borne out by anatomical findings) the chief symptoms are certainly not attributable to hypophyseal dysfunction but resemble those observed in acute experimental adrenal insufficiency. Failure to respond to treat-

ment with the adrenal cortical hormone in these cases may be more logically attributed to inadequate dosage or impotence of the preparations utilized.

The importance of the pituitary for normal adrenal function has been well established (Evans). The present work establishes the importance of the adrenal for maintaining those functions of the hypophysis which regulate normal growth, reproductive activity and thyroid activity. It need not be assumed that the adrenal elaborates a separate hormone necessary for proper hypophyseal activity, for our most highly purified adrenal cortical extracts suffice to maintain in adrenalectomized animals all the physiological functions attributed to the pituitary. It is only when these animals are subjected to an adrenal insufficiency over a sufficiently long period of time that the hypophysis is injured and produces manifestations which are also observed in acute adrenal insufficiency. It may be argued that these manifestations when observed during acute adrenal insufficiency are in reality also due to hypophyseal dysfunction attributable to the adrenal insufficiency. The improbability of this view is shown by the almost immediate cessation in growth of young rats following adrenalectomy, whereas growth does not cease for some weeks after hypophysectomy (Collip, Selye and Thompson). Moreover, we have not been able to induce growth in rats in acute adrenal insufficiency by the injection of potent extracts of pituitary growth hormone.

The present findings explain the apparent failure of some adrenalectomized animals to respond to adrenal cortical therapy after prolonged treatment. This apparent non-responsiveness is due to the use of an incomplete replacement therapy which, as we have seen, by producing a chronic adrenal insufficiency leads to a secondary hypophyseal deficiency. It is unnecessary to attribute the observed findings to the formation of antihormones as suggested by Collip.

SUMMARY

A state of chronic partial adrenal insufficiency was induced in rats, cats and dogs by various methods. The cessation of growth, failure of normal reproductive activity, and subnormal body temperature which ultimately manifested themselves in these animals were not remedied by adrenal cortical hormone therapy. On the other hand, injection of extracts of the hypophysis relieved the observed deficiencies. It is therefore concluded that hypophyseal insufficiency is induced by a chronic adrenal insufficiency and that this secondary hypophyseal dysfunction is responsible for the observed deficiencies.

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THE EFFECT OF INITIAL TENSION ON THE SPONTANEOUS ACTIVITY AND RESPONSES OF THE NON-PREGNANT CAT'S UTERUS

F. A. SIMEONE

From the Laboratories of Physiology in the Harvard Medical School

Received for publication April 8, 1935

Observations on the influence of initial tension on the responses of striated, cardiac and smooth muscles which contract in response to stimulation have shown that there is an optimum tension at which maximal contractions and liberation of energy occur (see Hampel, 1934, for references). The effects of initial tension on the responses of muscles which relax when stimulated have so far not been studied. It was deemed of interest to determine these effects. The non-pregnant cat's uterus, a muscle which relaxes in response to adrenine, was selected for study.

METHOD. Eight cats were used, under dial anesthesia. The body temperature was maintained constant by means of an electric heating pad. The uterus was approached through a midline abdominal incision. One horn was cut and freed after ligation of the ovarian vessels. The ovarian end was attached to a light writing lever and the vaginal end was fixed by a dissecting needle. The horn was enclosed in a glass cylinder to insure adequate moisture and temperature. Denervation of the uterus was obtained by severing the hypogastric nerves.

A weight pan was suspended from the writing lever at a distance of 2 cm. from the fulcrum, while the uterus was attached on the opposite arm at a point 1 cm. from the fulcrum. The weight of the pan was adjusted so that it just lifted the uterine horn to a vertical position under no detectable stretch. This was considered zero tension.

A dose of adrenine (commercial adrenalin) was selected which would insure a practically maximal response. Usually 0.5 cc., 1:50,000, was found suitable for this purpose. The responses to this dose injected intravenously were then recorded with weights increasing from 0 to 14 grams and repeated in the reverse order.

The base line of the records was complicated by the spontaneous rhythmic activity of the muscle. The responses were measured in centimeters on the record, from the mean of the rhythmic excursions for any given tension to the level of maximum relaxation.

RESULTS. *The effect of tension on the spontaneous rhythmic activity.*

Evans (1926) states that stretching smooth muscle renders it more excitable and institutes rhythmic contractions. He reports also that the greater the stretching force the greater the frequency of these contractions. In the present observations the degree of tension had no influence on the frequency of the rhythmic activity. Spontaneous contractions occurred

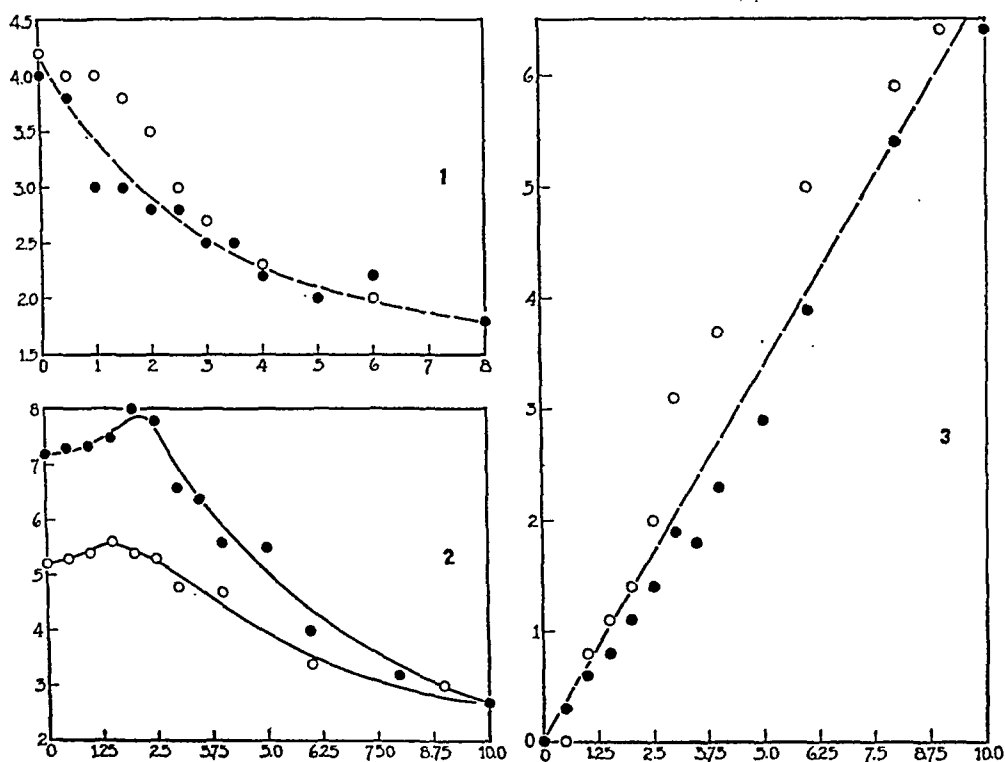


Fig. 1. Effects of tension on the spontaneous rhythmic activity of the uterus. Ordinates: amplitude of the contractions in centimeters in the record; magnification: 6. Abscissae: tension in grams. Dots: increasing tension. Circles: decreasing tension.

Fig. 2. Effects of tension on the responses to a maximal dose of adrenaline. Ordinates: relaxation in centimeters in the record; magnification: 6. Abscissae: tension in grams. Dots: increasing tension. Circles: decreasing tension.

Fig. 3. Increments of length as a function of tension. Ordinates: increments in centimeters $\times 6$. Abscissae: tension in grams. Dots: increasing tension. Circles: decreasing tension.

at zero tension and their amplitude diminished with increasing tension. Figure 1 illustrates a typical instance.

The effect of tension on the responses of the non-pregnant uterus to adrenaline. Observations on other types of muscle have shown an optimum initial tension for maximal response of the tissue to stimulation. The same relationship holds for the non-pregnant uterus in the cat. A maximal re-

sponse occurs with a load of 1 to 2 grams (effective load of about 1 gram). Figure 2 represents a typical instance. The increment of the responses at the optimum tensions was always slight but obtained consistently.

The influence of tension on the length of the uterus. Within the range of tensions used the mean length of the muscle was found to increase in an approximately lineal ratio with the tension, as shown in figure 3.

DISCUSSION. The purpose of this study was to obtain, if possible, some information regarding the nature of smooth muscle "tone" and the effects of tension thereon. Indeed, Ritchie (1928) has suggested that stretch is the stimulus responsible for "tone" in smooth muscle. The results reported do not support this hypothesis. If stretch caused contraction of smooth muscle we would expect contraction when tension is applied. On the other hand, tension would lengthen the muscle because of its elasticity. Either of these two antagonistic influences might preponderate. We actually find a lengthening (fig. 3), but a concealed stimulation is possible. A similar argument is applicable to the relaxations induced by adrenine.

The influence of tension on the magnitude of the spontaneous rhythmic activity, however, is against the view that "tone" is an effect of stretch. For, with low tension, the stimulus would be applied and the responses (amplitude of the rhythmic activity) should increase, both because of stimulation and because of optimum tension. Experimentally the amplitude of the rhythmic contractions decreases progressively with tension (fig. 1).

SUMMARY

Increasing initial tension causes a decrease in the amplitude of the spontaneous rhythmic contractions of the non-pregnant cat's uterus (fig. 1), while it has no effect on their frequency.

There is an optimum tension for the relaxations elicited by adrenine fig. (2).

The length of the uterus increases in an approximately lineal ratio with the tension (fig. 3).

The bearing of these data on smooth muscle "tone" is discussed.

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ALIMENTARY MOTOR CONDITIONING AND PITCH DISCRIMINATION IN DOGS

SIMON DWORKIN

From the Department of Physiology, McGill University, Montreal, Canada

Received for publication March 25, 1935

In previous experiments the writer (1935) established that the extreme limit of pitch discrimination in the cat is about one tone. This degree of sensitivity is considerably less than that found in the dog by Pavlov and his co-workers (see Andreyev, 1934). The difference in question may either be specific or might depend simply on the different effector responses used in the two cases. The cats had been tested by an alimentary-motor method. On the other hand, most of the workers on dogs have hitherto utilized the salivary reflexes. The main object of the present research was to measure the pitch-discriminating capacity of dogs when tested by the same alimentary-motor method as was applied to the cats. In some experiments, and in order better to compare the two procedures, simultaneous records of salivary and motor responses were taken from the dogs.

The experiments were carried out in a silence-room, under conditions identical with those of the experiments on cats (see Dworkin, 1934). Seven dogs were trained to respond by the motor method to various stimuli, and the limits of pitch discrimination were determined in two.

The mode of training was the same as that used with the cats. During a preliminary period the dogs were taught to obtain food for themselves by raising a hinged lid on the food-container. At a later period feeding was associated with specific stimuli, the latter being delivered, according to circumstances, by a bell, by a buzzer, by a light, by a tactile stimulation method, and by a low frequency pure-tone oscillator. The presentation of a stimulus and also the elicited response were in each case recorded graphically. The unconditioned (food) stimulus was a mixture of meat powder and boiled oatmeal.

Alimentary-motor conditioning. During early training all seven dogs were diffident and easily distracted. They took from one to three weeks to become accustomed to the room and to the conditions of the experiment. Only then were they willing to take food in the experimental chamber and to make attempts to raise the lid of the food-container. To the conditioning stimuli the latent period of some of the early responses was as long as

70 seconds. Once this hesitant or diffident stage was over, conditioned responses were developed easily and were well retained. Interval movement was never frequent, and if initially present, disappeared quickly. The minimum number of trials required to establish firm alimentary-motor responses depended partly on the strength of the stimulus and partly on the character of the animal. In the case of a pure tone of moderate loudness (40 to 50 decibels) conditioned responses could be well developed after 20 tests. A strong bell sometimes became effective after 4 to 5 trials, a tactile stimulus after 9 or 10 trials, while, with white light, responses were never elicited before 25 tests, and often not before 40 or 50 tests.

While the dog proves to be a good subject for alimentary-motor reflexes, it is far more liable than the cat to be affected by accidentally occurring stimuli. Complete inhibition was often produced by the sound of a door slammed near-by, by loud conversation in the experimenter's chamber, or even by some minor change in the arrangement of apparatus in the animal's chamber. On one occasion, after the position of certain battery cells which had hitherto stood in full view of the animals was changed, not merely the conditioned responses but even the eating of food was abolished for a time.

The limits of pitch discrimination. The two dogs used for experiments on pitch discrimination had been previously conditioned to respond to several types of stimulus. For the special tests, the procedure employed was that of "differentiating inhibition" (Pavlov). In the course of its six or seven daily positive tests one negative (not reinforced by food) stimulus was used. As far as possible the loudness of the notes to be differentiated was—in the manner described elsewhere (Dworkin, 1935)—kept equal.

In the case of one dog a note of 2500 cycles per second was chosen as a positive stimulus, one of 3500 cycles as the first negative stimulus. The duration of the negative stimulus was 20 seconds. After 20 trials, occasional discriminations were noted, and after 30 trials the discrimination was complete (zero response to several successive negative stimuli). The negative stimulus was then changed to 3000 cycles per second. This was distinguished from 2500 cycles at the 34th trial. Thereafter 2900 cycles was promptly differentiated. The positive tone was then changed to 2600 cycles. This was differentiated from 2900 cycles at the 40th trial. At the 54th trial, 2700 cycles was distinguished from 2900 cycles, and at the 61st trial, from 2830. The limit of firm discrimination in this dog, attained after 83 trials, proved to lie between 2775 and 2700 cycles per second. This represents an interval of about one-third of a tone. After several more negative tests in attempts to form a closer differentiation, complete inhibition resulted, first to the conditioned, then to the unconditioned stimuli.

The second dog discriminated between 2700 cycles and 2900 cycles in 67 tests, and between 2820 and 2900 cycles in 83 tests. With this animal, too, it proved impossible to develop further differentiation.

These limiting determinations—representing intervals of about one-third of a tone—are quite in agreement with those observed by some of the Russian workers (Zeliony, 1907; Anrep, 1920; Andreyev, 1934). It may therefore be concluded that the dog does really have a greater capacity for pitch discrimination than the cat.

*Direct comparison of the salivary and the motor responses.*¹ It had thus appeared that, in the matter of pitch discrimination, there is general agreement between the results obtained by the motor method and those obtained by the salivary method of the Russian workers. In order to compare the two methods still further, it was decided to take simultaneous records of the salivary and the motor reactions to specific positive and negative stimuli.

Only after the motor responses to the relevant stimuli were well established did one proceed to record the salivary flow. By means of a metal or glass cup fastened to the surrounding skin saliva was collected from a parotid fistula. To record the flow electrically, a water-filled system and vacuum-tube-operated relay were used. For proper operation of the relay, strong suction must be applied to the liquid in the system. This in turn calls for an air-tight seal between the cup and the skin surface. Whether with Mendeleeff's cement, with collodion, or with several other adhesive materials tried, it proved somewhat difficult to obtain a satisfactory seal. When it was desired simply to measure the saliva by displacement of fluid in a horizontal manometer, the Mendeleeff cement did, however, afford an adequate joint.

Typical results from some of the successful experiments are shown in figure 1. In general, it may be said that the two methods give equivalent results. Nevertheless, certain differences between the motor and salivary responses were observed. One might best select for comparative comment the latent period, the duration of the response, interval activity and inhibition.

Latent period. Usually both responses occurred simultaneously, though in many instances one preceded the other by some seconds. Records A, B and C of figure 1 show how the time relationship may vary. By way of additional illustration a representative protocol of successive responses to an oscillator-tone stimulus may here be cited.

¹ Some of these experiments were carried out with the kind collaboration of Dr. G. F. Sutherland.

Dog "Whitey" April 26, 1934

TIME	TEST NUMBER	STIMULUS	LATENT PERIOD	
			Motor	Salivary
			<i>seconds</i>	<i>seconds</i>
11:41 a.m.	657	2000 cycles	2-3	12-13
11:44 a.m.	658	2000 cycles	2	1.5
11:49 a.m.	659	2000 cycles	2.5	3.5
11:52 a.m.	660	2000 cycles	2	2.2
11:58 a.m.	661	2000 cycles	2.5	2
12:02 p.m.	662	2000 cycles	2.5	4.5
12:05 p.m.	663	2000 cycles	2.5	2.5

Duration of response. Pavlov has divided the salivary reaction into two phases, conditioned and unconditioned. The conditioned saliva is that secreted between the beginning of the stimulus and the presentation of the food. The unconditioned is that which commences when the animal has food in its mouth. These two phases appear separately in the first five records of figure 1.

The recorded motor response is in its nature somewhat different. It sharply exhibits two things, the moment of opening and the moment of closure of the lid. The opening of the lid may be considered as the physiological equivalent of the conditioned flow of saliva. What is noteworthy is that the dog invariably drops the lid before the unconditioned flow of saliva has ceased. In other words, the duration of the recorded motor response is always less than that of the whole salivary flow, which thereafter goes on for a time, to die off gradually.

Interval activity. As already stated, interval motor response rarely occurs in a well trained dog, yet nothing in the way of punishment or physical hindrance is employed to prevent it. When interval movement did occur, it was usually, but not always, accompanied by saliva. Interval saliva was, however, often secreted without motor activity (see fig. 1, F). Furthermore, weak intercurrent stimuli could elicit salivary flow without motor response (fig. 1, F). These results may possibly mean that the threshold for salivary reflex is somewhat lower than for the motor response.

Inhibition. In establishing differentiations, it was noted that the motor responses to the negative stimuli were abolished earlier than the salivary responses. This point is illustrated in figure 1, D and E. A note of 2500 cycles was the negative (delayed) stimulus. At the 17th negative trial two separate, very brief, conditioned motor responses were elicited; the salivary flow began simultaneously with the first motor response and was likewise discontinuous (fig. 1, D). At the 19th negative trial the conditioned motor response was abolished, yet saliva began to flow after a

latent period of 7 seconds, and was, for a time, copious (fig. 1, E). After 30 negative tests the salivary reflex, too, was abolished.

In cases when, after a positive stimulus, the presentation of the food was delayed, the animal dropped the lid to pick it up again once or twice. A precisely parallel discontinuity was often observed in the salivary secretion.

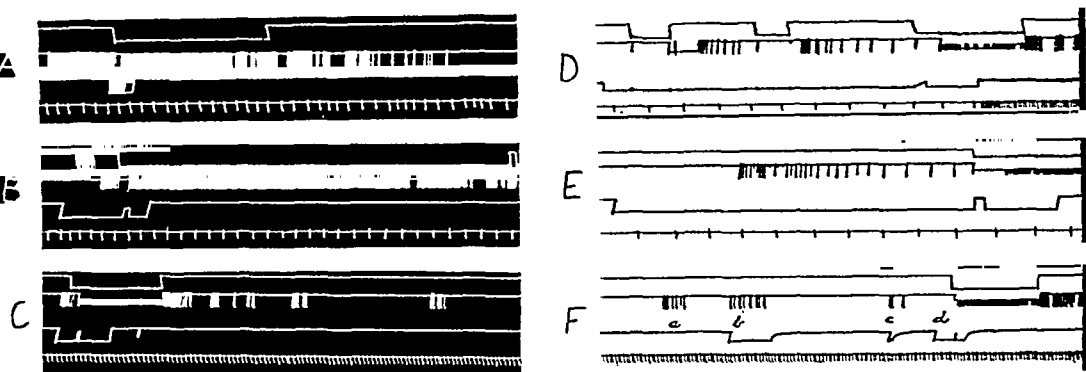


Fig. 1. Six examples of simultaneous records of motor and salivary responses. Read from left to right.

In each case, the top line represents the motor response (lifting and dropping of the lid). The second line represents the salivary flow, in vertical lines. The third line represents the stimulus, while the presentation of the food is indicated by the break in this record. The bottom line represents time in two-second intervals. The kymograph speed was not the same in all experiments.

In record A the motor and salivary responses begin simultaneously; in record B the motor precedes the salivary by 4 seconds, and in record C the salivary precedes the motor by 3 seconds. Whatever the latent period, the total duration of the salivary response always exceeds that of the motor response.

Records D and E show the effect of inhibition (in this case delay) upon the two reflexes. In D the motor response is brief and discontinuous, while a moderate amount of conditioned saliva is secreted. In E the motor response is abolished, while conditioned saliva is still elicited.

In record F interval saliva is shown at *a*, without motor response. At *b* and again at *c* a weak buzzer was sounded. Saliva was secreted, but the lid was not opened. At *d* the regular stimulus was turned on and elicited both responses promptly.

CONCLUSIONS

1. The dog is a good subject for alimentary-motor conditioned reflexes. In the case of this animal, however, special precautions must be taken to avoid the inhibitory effects of extraneous stimuli.

2. The capacity for pitch discrimination of pure tones in the dog is about one-third of a tone as against one whole tone in the cat. This indicates the possession of a superior acoustic analyser by the dog.

3. Simultaneous records of motor and salivary responses to specific positive and negative stimuli show similar end-results. The responses may differ, however, in respect of latent period, duration, interval activity and inhibition.

For financial assistance in this work, the author wishes to acknowledge his indebtedness to the Research Bureau of the American Otological Society.

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ELECTRICAL STIMULATION OF THE INTERIOR OF THE CEREBELLUM IN THE MONKEY¹

H. W. MAGOUN, W. K. HARE AND S. W. RANSON

From the Institute of Neurology, Northwestern University Medical School

Received for publication March 11, 1935

Investigations of the cerebellar nuclei and the white matter adjacent to them have been very few in number as compared with many studies of cerebellar function based upon gross extirpation of this organ or upon destruction or stimulation of its cortex. It is well known, however, that the nuclei constitute at least the major source of all efferent cerebellar connections, and the small amount of attention which they have received is by no means commensurate with their importance in cerebellar activity. The difficulty of approaching these nuclei, situated as they are within the interior of the cerebellum, has doubtless been the primary obstacle to their study, but this difficulty has been overcome by several investigators.

With the immediate object of investigating the reactivity of the cerebellar nuclei to electrical stimulation, Horsley and Clarke (1) devised their stereotaxic instrument for accurately orienting an electrode within the interior of the brain. Unfortunately their study of the cerebellar nuclei was never completed, and we have only brief and incidental mention of their results (1, 2). At Horsley's invitation this work was continued by Sachs and Fincher (3) but only a preliminary report was made, in which again but brief mention of the results appeared. Some observations of the effect of electrical stimulation of the medial cerebellar nuclei, with the aid of the Horsley-Clarke instrument, have been reported by Mussen (4). In the work of Miller and Laughton (5, 6) on the decerebrate animal, the cerebellar nuclei were electrically stimulated after their exposure by ablating the over-lying cerebellar parts.

In the present investigation, the Horsley-Clarke stereotaxic instrument has been employed in the electrical stimulation of the cerebellar nuclei and the territory adjacent to them in the rhesus monkey (*Macaca mulatta*).

METHOD. Stimulation of the cerebellum was performed under light nembutal anesthesia (10-19 mgm. per kgm. of body weight) plus supplemental ether added when necessary by means of a tracheal cannula and ether bottle. The animal was suspended untied in a hammock with the limbs hanging free and the head in the freely swinging stereotaxic instru-

¹ Aided by a grant from the Rockefeller Foundation.

ment supported from above. In some of the experiments, the body was supported from above by strings under the supraspinous ligament at the shoulder and pelvis. The technique of electrical stimulation used has been described in detail by Ranson (7), and need be only briefly referred to here.

Using the Horsley-Clarke stereotaxic instrument and a bipolar needle-like electrode, less than 1 mm. in diameter, with the tips of the two wires separated by a distance of 1 mm. along the axis of the electrode, the interior of the cerebellum was systematically explored by electrically stimulating in orderly succession every cubic millimeter of its substance. The current was supplied by a single dry cell registering 1.5 amp. attached to a Harvard inductorium, the secondary coil of which was set at 9 cm.

In eight monkeys stimulation was performed along punctures in a vertical plane, the electrode being inserted through the overlying cerebral hemisphere, after removal of a part of the calvarium and dura, and retraction of the superior sagittal sinus. In four of these animals, stimulation was begun on the left side and extended to the midline and onto the right side. In the other four, stimulation was begun to the right of the midline and extended onto the left side. In two cases the exposure was made through the occipital bone above the foramen magnum, and stimulation of the left side was performed along punctures in a horizontal plane, the electrode being introduced through the caudal, rather than the dorsal, surface of the cerebellum. In every case, stimulation was begun in the caudal portion of the cerebellum and continued rostrally through the cerebellar nuclei to the cerebellar peduncles. The intact efferent cerebellar pathways, therefore, always lay ahead of every point of stimulation.

Subsequent microscopic examination of serial sections of the explored area enabled the localization of each point stimulated. A correlation between the location of these points and the responses obtained from their stimulation, as represented by appropriately situated symbols on a series of eight transverse sections at millimeter intervals through the interior of the cerebellum, gave a composite and graphic picture of the results obtained.

RESULTS. It is interesting to note that in the order of frequency of response to cerebellar stimulation, the parts of the body have been represented in this series of experiments as follows: eyes, ipsilateral forelimb, head, contralateral forelimb, hind limbs, trunk and tail. In our opinion no particular significance should be attached to this sequence, beyond the suggestion that the anterior part of the body has a greater representation than the posterior.

The responses of the eyes, head, trunk and tail have consisted of deviations from the longitudinal axis of the body and may be grouped together as reactions of the eyes, head and axial musculature. The responses of

the limbs form a second group manifest as reactions of the musculature of the extremities. For convenience in presenting the results, the responses of the two groups may be taken up separately, but reactions of both groups have occurred together from stimulation of certain regions of the cerebellum.

The responses obtained from cerebellar stimulation have been definitely postural reactions, as distinct from the quick reactions produced by stimulation of the cortico-spinal system or peripheral nerves, and from the usually phasic responses obtained through reflex activation of the motor system. Such cerebellar responses take a number of forms. They may consist of a slow movement during stimulation to a posture which is maintained throughout stimulation. On the other hand, an inhibition or relaxation of a previously existing muscular contraction often occurs during stimulation. After stimulation, there follows either a sudden rebound to a posture usually representing the opposite of that produced by stimulation, or a slow assumption of an attitude, usually after repeated stimulation, which may persist as a postural background throughout the experiment.

Eyes, head and axial musculature. The reactions of the eyes, head and axial musculature to electrical stimulation varied with the region stimulated and consisted: first, of a marked conjugate deviation of the eyes and a slight turning of the head (not always present) to the side stimulated, or secondly, of a movement of the eyes and usually the head to a position of forward gaze, each from a position of contralateral deviation existing as a posture slowly assumed during the course of the experiment. In every case these reactions of the first and second type were confined to the eyes and head. Responses of these types have been very widespread throughout the interior of the cerebellum, being obtained from the stimulation of points within the extent of all of the cerebellar nuclei, and the white matter adjacent to them.

Reactions of a third type have concerned not only the eyes and head, but in some instances the trunk and tail also. In the latter case, they represent responses of the entire length of the body axis. These reactions of the third type were made up of two phases, the first occurring during the period of stimulation, and the second occurring as a rebound at the end of stimulation. During the period of stimulation, the eyes and head moved to a position of forward gaze or deviated to the side of stimulation, as before. Immediately following the cessation of the stimulus, the eyes and head briskly and actively deviated to the contralateral side.

Such responses of the head are shown in some of the photographs of figures 1 and 2. In figure 1, A and B, the head was turned slightly to the left during stimulation of the left side of the cerebellum (A), and at the end of stimulation exhibited a rebound deviation to the right (B). In figure

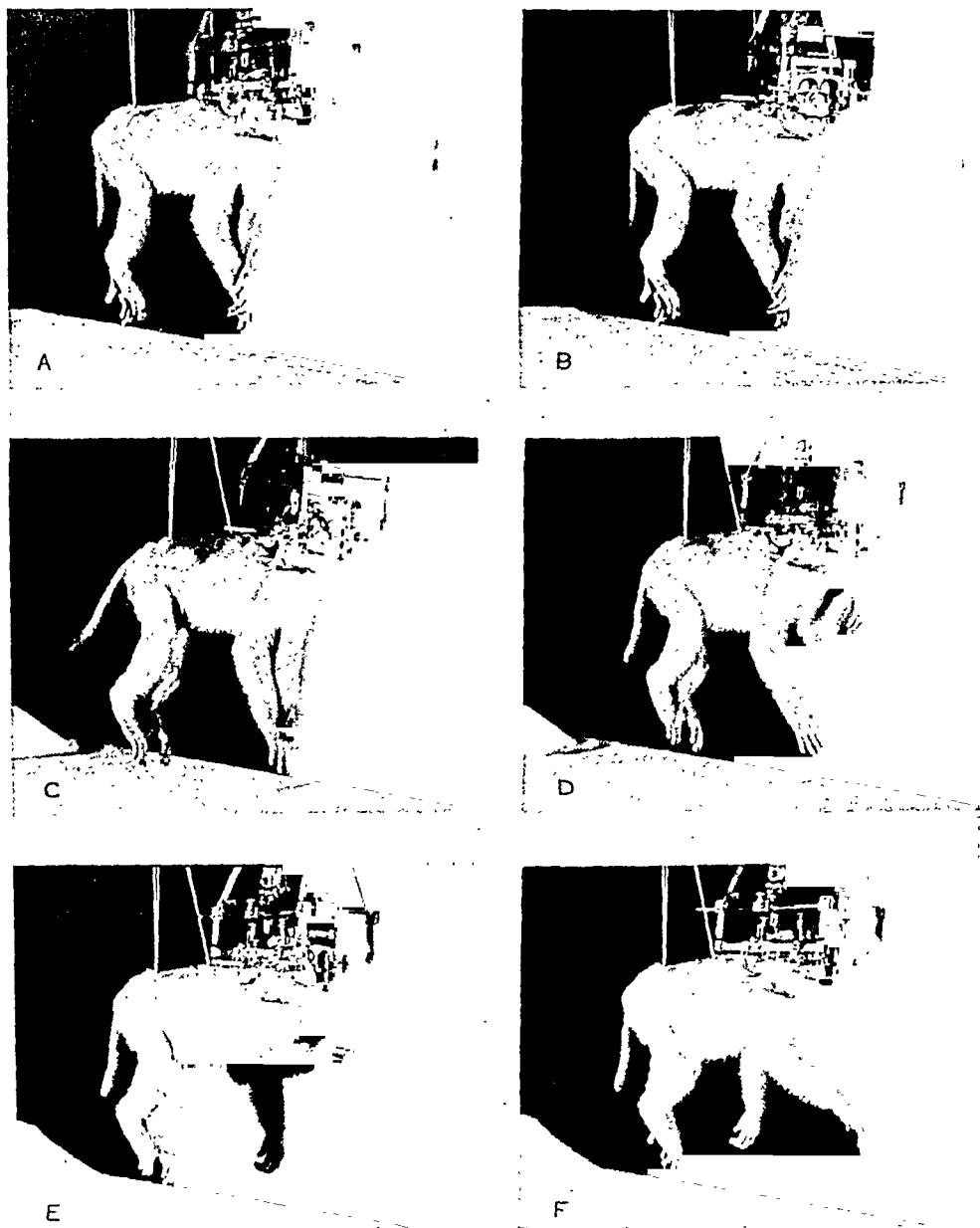


Fig. 1 is made up of three pairs of photographs, each pair showing the responses to stimulation of a single point within the interior of the cerebellum. In each case, the photograph on the left shows the phase of the reaction occurring during stimulation, the photograph on the right the rebound after stimulation. Photographs A and B show a response of the head, stimulation being on the left side of the cerebellum. Photographs C and D show a response of the ipsilateral forelimb, stimulation being on the left side of the cerebellum. Photographs E and F show a response of the ipsilateral forelimb, stimulation being on the right side of the cerebellum. The animals were under nembutal anesthesia and did not experience pain.

2, E and F, the head was turned to the right during stimulation of the right side of the cerebellum (E), and exhibited a rebound deviation to the left at the end of stimulation (F). In figure 2, C and D, the head took a position of forward gaze during stimulation of the right side of the cerebellum (C), and exhibited a rebound deviation to the left at the end of stimulation (D). In the photographs movements of the head can be gauged best by noting changes in the long axis of the stereotaxic instrument.

Responses of the trunk and tail, when present, occurred in two phases. The first phase appeared during stimulation and consisted either of a relaxation of a preëxisting concavity of the body axis to the opposite side, or of the production of a concavity of the trunk and deviation of the tail to the side of cerebellar stimulus. The second phase appeared as a rebound at the end of stimulation and consisted of the production of a concavity of the trunk and a deviation of the tail to the side opposite cerebellar stimulus.

Responses of the body axis do not stand out clearly in the accompanying photographs, but were present in each of the responses shown in figure 2. In figure 2, A and B, the body axis was straightened during stimulation of the left side of the cerebellum (A), and exhibited a rebound concavity to the right at the end of stimulation (B). In figure 2, C and D, the body axis was straightened during stimulation of the right side of the cerebellum (C), and exhibited a rebound concavity to the left at the end of stimulation (D). In figure 2, E and F, the body axis exhibited a concavity to the right during stimulation of the right side of the cerebellum (E), and a rebound concavity to the left at the end of stimulation (F).

These responses of the third type were obtained only from the medial part of the interior of the cerebellum, i.e., from the stimulation of points in the white matter bordering on the roof nuclei, and in some instances also from points on the medial edge of the emboliform nucleus.

Limbs. Postural reactions of the limbs have been obtained both as responses during stimulation and as rebound contractions at the end of stimulation. Two groups of such responses may be differentiated.

Group I of the limb responses includes a number of reactions, usually slight in excursion and confined to parts of the ipsilateral forelimb, or rarely to the ipsilateral hind paw. These responses consisted of two phases and usually made their appearance as a brisk, short-lasting assumption of posture at the end of the period of stimulation. A second stimulation, immediately following, relaxed this posture, but at the conclusion of the stimulus, the attitude was once more assumed. Because of its appearance at the end of stimulation, the assumption of posture appeared to be of the nature of a rebound contraction following inhibition. As a common variation, an attitude was often assumed as a result of a contraction of one muscle group during the period of stimulation, while a brisk rebound con-

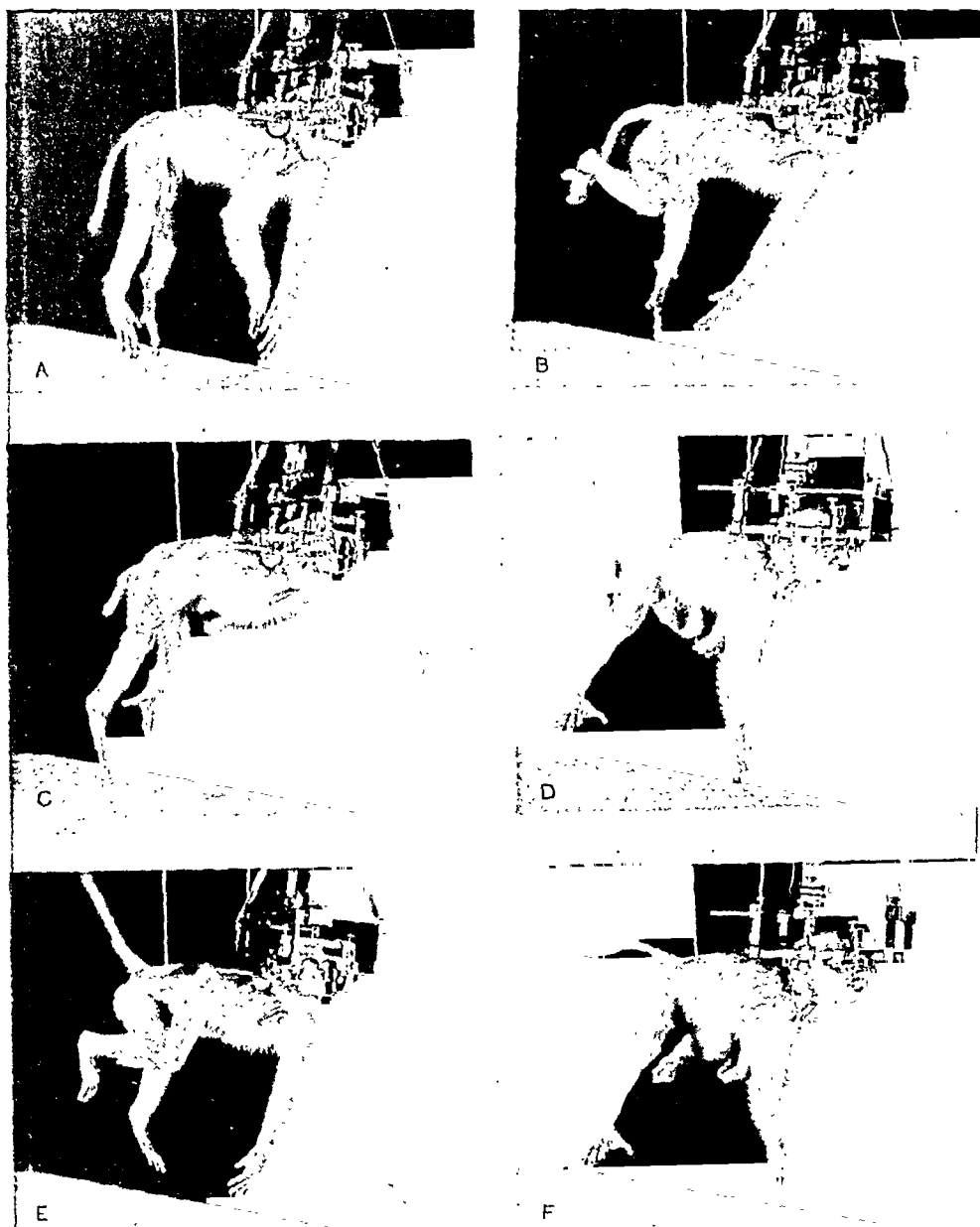


Fig. 2 is made up of three pairs of photographs, each pair showing the response to stimulation of a single point within the interior of the cerebellum. In each case, the photograph on the left shows the phase of the reaction occurring during stimulation, the photograph on the right the rebound after stimulation. Photographs A and B show responses of the limbs and body axis, stimulation being on the left side of the cerebellum. Photographs C and D show responses of the limbs and body axis, stimulation being on the right side of the cerebellum close to the midline. Photographs E and F show responses of the limbs and body axis, stimulation being on the right side of the cerebellum. The animals were under nembutal anesthesia and did not experience pain.

traction of the respective antagonistic muscles at the end of stimulus reversed the attitude in the part concerned. In none of these cases was there a rebound rigidity or resistance to passive manipulation.

Responses of this nature consisted: *a*, of a flexion or relaxation from extension of the forearm, hand, or fingers during the period of stimulation, followed at the end of stimulation by a rebound extension of these parts of the limb; *b*, of a relaxation from flexion during stimulation followed by a rebound flexion of these parts at the end of stimulus; *c*, of a relaxation from pronation or of an active supination of the forearm and hand during the period of stimulation, followed by a rebound pronation at the end of stimulus; *d*, of a relaxation from abduction or an active adduction of the arm during the period of stimulation, followed at the end of stimulus by a rebound abduction. These responses appeared of a diverse nature from the point of view of the muscles and joints involved, but they could be associated in that they were all concerned with postures of parts of the ipsilateral anterior extremity.

Photographs of two reactions belonging to this group are shown in figure 1. In figure 1, C and D, a response is shown of the left forelimb, stimulation being on the left side of the cerebellum. During stimulation the left forelimb was relaxed (C), while at the end of stimulation it exhibited a rebound flexion of the elbow and wrist (D). In figure 1, E and F, a response is shown of the right forelimb, stimulation being on the right side of the cerebellum. During stimulation the right forelimb was flexed at the elbow and slightly supinated (E), while at the end of stimulation it exhibited a rebound extension of the elbow and some pronation of the forearm (F).

Responses of group I were obtained from the buried cerebellar cortex of the anterior lobe both caudal to and overlying the fastigial, globose, and emboliform nuclei. The reactive points converged laterally in the underlying white matter, so that many responses were obtained from the stimulation of points in the region occupied by the emboliform and globose nuclei, while no responses of this nature were obtained from the dentate or fastigial nuclei.

Group II consisted of responses of all four limbs. These reactions were similar to those just described in that the reaction occurred in two phases, and was usually first seen as a rebound assumption of posture at the end of the period of stimulation. A second stimulus relaxed this posture, which was briskly assumed once more as the stimulus was concluded. While a number of variations were observed in the postures involved in the responses of this group, very commonly the reactions consisted of a relaxation of the limbs during stimulation, followed, at the end of stimulus, by a brisk and gross rebound extension of the limbs on the ipsilateral side and a flexion of those on the contralateral side. The rebound was usually

greater in the forelimbs than in the hind and was often most marked in the ipsilateral forelimb. In the marked responses of this nature, the rebound posture was maintained for a period of minutes, as a strong rigidity and resistance to passive manipulation, chiefly of the forelimbs and greatest in the ipsilateral forelimb. In a number of cases such rebound postures were timed and were still undiminished at the end of five minutes. The cycle of inhibition and rebound could be continued indefinitely with repeated stimulation, the responses becoming augmented on repetition.

In some cases, instead of a relaxation of a preëxisting posture during stimulation, an actual contraction occurred in the muscle groups antagonistic to those contracting in the rebound at the end of stimulation. That is, during stimulation there was a flexion of the ipsilateral limbs and an extension of the contralateral limbs, while after stimulation there followed, as before, a rebound extension of the ipsilateral limbs and a flexion of the contralateral limbs. In reactions obtained from stimuli close to the midline, the limbs of the two sides often assumed similar rather than contrary postures, either during or after stimulation.

In whatever form they were manifest the reactions of group II appeared to be concerned not so much with the attitudes seen in small movements of specific parts of a single anterior extremity, as with those involved in the gross postures of all four limbs.

Photographs of reactions belonging to this group are shown in figure 2. In figure 2, A and B, a response is shown to stimulation on the left side of the cerebellum. During stimulation there was a relaxation of all limbs (A), which was followed at the end of stimulation by a rebound extension of the left limbs and a rebound flexion of the right limbs (B). This rebound posture (B) was timed, and at the end of five minutes showed no appreciable relaxation.

Figure 2, C and D, shows a response to stimulation of the right side of the cerebellum, close to the midline. During stimulation both forelimbs were retracted and flexed (C). At the end of stimulation there was a rebound extension of the right limbs and a rebound flexion of the left limbs (D).

Figure 2, E and F, shows a response to stimulation of the right side of the cerebellum. During stimulation the right limbs were flexed and the left limbs were extended (E). At the end of stimulation the right limbs exhibited a rebound extension and the left limbs a rebound flexion (F). The photograph of the rebound posture (F) was taken two minutes after the cessation of the stimulus. A comparison of the rebound postures shown in figure 2, D and F, with that obtained from the opposite side of the cerebellum (fig. 2, B) will demonstrate the striking reversal of response encountered in passing from one side of the midline to the other, reactions of contrary sides being mirror images of one another. A similar reversal

on crossing the midline is seen in the movements of the head. During stimulation of appropriate points on the left side, the head turns to the left and after stimulation rebounds to the right (fig. 1, A and B). Conversely, stimulation on the right side produces a turning of the head to the right during stimulation followed by rebound to the left (fig. 2, E and F).

Reactions of group II were obtained from the buried cerebellar cortex of the anterior lobe, both caudal to and overlying the globose and fastigial nuclei. The reactive points appeared to converge slightly medially in the underlying white matter, so that many responses were obtained from the stimulation of points in the vicinity of and within the substance of the fastigial nucleus, while at least the caudal part of the globose nucleus appeared definitely lateralward of the responsive region. No responses of this nature were obtained from the emboliform or dentate nuclei.

The reactions which have been reported above, from stimulation of the interior of the cerebellum, disappeared as the electrode emerged from the lower surface of the cerebellum into the fourth ventricle. Stimulation of points in the underlying brain stem never has yielded responses of the types described above, but chiefly contractions of isolated groups of muscles supplied by the fifth, sixth, seventh or eleventh cranial nerves.

DISCUSSION. The results just reported indicate the extent to which this method of electrical stimulation may be employed in an investigation of the interior of the cerebellum. The technique does not appear to be applicable to a study of all parts of this region. For example, stimulation of points within the substance of the dentate nucleus, under the conditions of these experiments, has yielded no consistent response specific to this nucleus. There are, however, two regions of the interior of the cerebellum which, in these experiments, have been regularly associated with a consistent type of reaction pattern.

The first of these is the region of the emboliform and globose nuclei and the neighboring white matter, which appears to be closely associated with the limb responses of group I, concerned with small and short-lasting postures of parts of the ipsilateral anterior extremity. The second is the region of the fastigial nucleus and the neighboring white matter, which appears to be closely associated with the limb responses of group II, concerned with gross and long-lasting postures of all four limbs, and probably also with the eye, head, and trunk responses of type III, concerned with lateral deviations from the longitudinal body axis.

The biphasic nature of these responses, consisting usually of an inhibition during stimulation and a rebound contraction after stimulation, suggests their relationship to similar responses obtained by a series of workers from stimulation of the cerebellar cortex in the decerebrate preparation. Reference has been made to a number of these studies in the most recent

report on this subject, that by Denny-Brown, Eccles, and Liddell (8). There are many points of similarity between the results obtained by these authors and our observations on the limb responses of group II, which in this series of experiments have been followed from the buried cortex of the anterior lobe through the underlying white matter to the region of the fastigial nucleus.

This similarity suggests that we are dealing here with one and the same reaction, obtained by Denny-Brown, Eccles, and Liddell from stimulation of the surface of the cerebellar cortex, and obtained in the present investigation from stimulation of the underlying cortex and white matter, and region of the fastigial nucleus. If this be true, the presence or absence of a decerebrate rigidity in the animal would appear to be an indifferent factor, possibly serving only to quantitatively augment the normal reaction of this cerebellar system to the point at which it might be elicited by surface stimulation of the cerebellar cortex.

This suggestion receives support from the fact that the results of the present study are in agreement, in some general features, with the observations of Miller and Laughton (5, 6) obtained from stimulation of the cerebellar nuclei in the decerebrate cat.

SUMMARY

Electrical stimulation of the interior of the cerebellum in the monkey, employing the Horsley-Clarke stereotaxic instrument, has yielded responses of the eyes, head, and axial musculature, and responses of the limbs.

With the possible exception of some reactions of conjugate deviation of the eyes and head to the side of stimulation, these responses have been biphasic in nature, and have consisted of one effect during the period of stimulation, followed by a second effect occurring as a rebound at the end of stimulation.

Such responses have consisted either of an inhibition of a muscle group during stimulation followed by a rebound contraction of this muscle group at the end of stimulus, or of a contraction of one muscle group during stimulation followed at the end of stimulus by a rebound contraction of its antagonist.

Reactions of this nature involving small and short-lasting postures of parts of the ipsilateral anterior extremity have been traced from the buried cortex of the anterior lobe through the underlying white matter to the region of the emboliform and globose nuclei.

Other reactions of this nature involving pronounced and long-lasting postures of all four limbs, the rebound contractions usually persisting for a period of minutes, have been traced from the buried cortex of the anterior lobe through the underlying white matter to the region of the fastigial nucleus. Similar responses of the eyes, head, and axial musculature, in-

volving lateral deviations from the longitudinal body axis, have been obtained from the white matter in the vicinity of the fastigial nucleus.

Somewhat comparable reactions have been observed from cerebellar stimulation in the decerebrate preparation by a series of workers. The suggestion is made that such reactions simply represent normal cerebellar responses, the presence of a decerebrate rigidity being an incidental factor.

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THE QUANTITATIVE DETERMINATION OF URINARY OESTRIN

GEORGE VAN S. SMITH AND O. WATKINS SMITH

*From the Fearing Research Laboratory, Free Hospital for Women, Brookline, Mass.
The Mrs. William Lowell Putnam investigation of the toxemias of pregnancy*

Received for publication February 28, 1935

In analyzing pregnancy urines for oestrin so much of the hormone is present that extraction is not necessary. We have therefore assumed that the results upon slightly acidified unextracted specimens from this source were as quantitatively accurate as could be expected in any determinations in which a biological means of assay must be used. In the case of urines from non-pregnant individuals, however, with which concentration is called for, the striking inconsistency of the results of the different recognized methods of extraction has convinced us of their quantitative inadequacy. We therefore set out, by experiments on assayed pregnancy urines, to test a number of solvents with the purpose of finding some method that would give complete recovery of urinary oestrin. The outcome of these experiments has forced us to the conclusion that all of our previous so-called quantitative values on both pregnant and non-pregnant individuals represent a variable and in most instances only a small part of the oestrin that we have since been able to obtain. Moreover, they are not comparable from a quantitative standpoint and are significant only in that a large number of analyses may have roughly indicated certain general trends.

In all of the experiments to be reported the urine specimens were acidified when received, if necessary, with dilute hydrochloric acid to a pH between 7.0 and 4.0, i.e., acid to litmus but not to congo red paper. Most of the specimens were at this pH when received. No preservative was added but the urines were kept cold and the pH always checked before assay. All extracts were taken up in olive oil unless otherwise specified, since this medium has been found to give slightly higher values than normal saline solution. The technique of assay has been that of Kahnt and Doisy, with rats which had been previously standardized (5). Whenever possible, at least 10 rats have been used in determining the minimum dose, but with the few urines of non-pregnant women which contained less than 5 rat units per 24-hour amount, it was necessary to depend for the result on 3 animals. Twenty-four-hour urines were always collected and the amount of oestrin expressed in terms of rat units per 24-hour volume.

Four different solvents were first tested upon 8 urine specimens from pregnant women and the results compared with assays of the unextracted material. Chloroform, either by continuous extraction according to Frank (1) or by repeated reflux extraction, yielded 13 to 25 per cent as much oestrin. By olive oil extraction according to Doisy et al. (2) 35 to 42 per cent was recovered. Ethyl acetate (3) gave 30 to 60 per cent and continuous 24-hour extraction with benzene¹ in the apparatus shown in figure 1 recovered 72 to 96 per cent of the oestrin found in the particular 8 specimens before extraction. The percentage yields for each of these solvents varied over such wide limits as to make it evident that no one of them would produce even comparable figures. Moreover, when 12 additional pregnancy urines were extracted by the benzene method, the extracts of 7 of them contained up to 5 times as much as was demonstrable by testing the straight urine. Apparently our original assumption, that assays on unextracted urines could be used as a gauge of the amount of oestrin actually present, was erroneous. Some oestrin must be bound in certain specimens by other urinary constituents and is at least partially freed by the process of extraction. But we could not conclude that benzene freed and recovered all the oestrin present, since in the other 5 instances less was in the extract than in the urine. Neither decomposition nor changes in pH could be found accountable for the inconsistent results. It was apparent that, in order to get true or even comparable values, some method of "freeing" all the hormone before assay or extraction would be necessary.

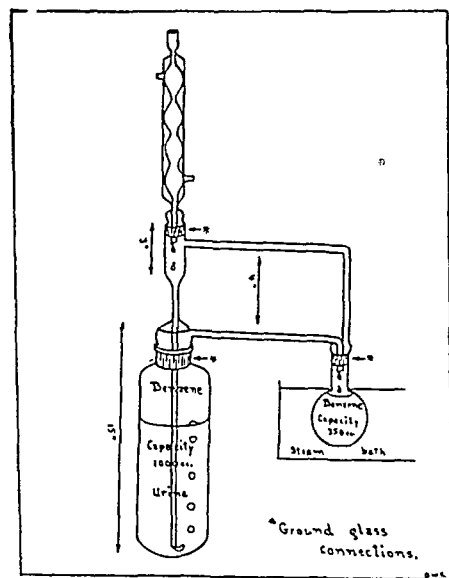


Fig. 1. Apparatus for continuous extraction of urinary oestrin with benzene.

The boiling of the urine of pregnant mares with concentrated hydrochloric acid yields values about 3 times as high as those given by the untreated urine (6). Zondek (8) claims that no preliminary acid treatment is required for the complete removal of oestrin from human pregnancy urines by extraction, but others (2, 9, 10) have found that the oestrin

¹ Merck's benzene (benzol)—reagent. Siebke (4) employs benzene and recommends reflux extraction. In the only instance in which we tried his method we obtained no greater yield than with 24-hour continuous extraction.

obtainable from this source may be augmented by preliminary acid treatment. Since Lipschutz and Poch (6) state that the greatest increase is produced by boiling for 5 minutes with 10 to 20 per cent by volume of concentrated hydrochloric acid, we adopted their procedure. To each 100 cc. of urine, therefore, 15 cc. of concentrated hydrochloric acid were added and the mixture boiled vigorously for 5 to 10 minutes. In the case of ethyl acetate it was necessary to neutralize the urine again to a pH between 7 and 4 before extraction, since otherwise acetic acid is formed. This neutralization does not cause any demonstrable loss of the increased potency *provided the extraction is carried out immediately*. The fact that neutralization is not called for before extracting with benzene further recommends this solvent.

Table 1 presents figures for the comparative yields from untreated and acid treated urines of pregnant women. For the sake of simplicity we shall call the yield from untreated slightly acid urines "free oestrin" and that from urines boiled with hydrochloric acid "total oestrin." The term "total" may be misleading. Although, as will be shown below, no other procedures that we have tried have yielded more oestrin than has 5- to 10-minute boiling with 15 per cent hydrochloric acid, it is possible that some other process might recover or produce even more of the hormone. An increase in the amount of oestrin in all of these specimens followed boiling with hydrochloric acid. The ratios between unextracted "total" and unextracted "free" oestrin, however, vary from 1.5 to 1 (urine no. 7 (b)) to 40 to 1 (urine no. 5(c)), indicating that the "free" oestrin is probably dependent upon variations in the urinary constituents which cause the hormone to be partially inactivated or bound. The figures for "total" oestrin, on the other hand, are as quantitatively satisfactory as could be expected; viz., the amounts in the saline benzene extracts of treated urines check very well with the amounts found in unextracted specimens after boiling with hydrochloric acid, proving complete recovery by benzene. The olive oil benzene extracts rendered the values slightly higher in some instances, due probably to slower and more uniform absorption. Ethyl acetate extraction is not as complete as benzene but, when applied to urines that have been boiled with acid, probably gives roughly comparable figures.

It still remained to be confirmed that 5- to 10-minute boiling with 15 per cent acid gave as complete a yield as was possible by acid treatment. Eleven samples of a pregnancy urine were treated with different amounts of hydrochloric acid and heated for varied periods. The procedures and results are summarized in table 2. It was concluded that in order to obtain the greatest oestrogenic potency without any destruction, urine should be boiled for 5 to 60 minutes after adding 15 to 30 per cent by volume of

TABLE 1

Demonstrating the augmented oestrogenic potency of human pregnancy urines after boiling with acid, and the quantitative recovery of "total" oestrin by benzene extraction

SOURCE OF SPECIMEN	OESTRIN RECOVERY—RAT UNITS IN 24° VOLUME					
	Untreated (pH of 7.0 to 4.0) "Free"			Boiled with 15 per cent HCl—"Total"		
	Unex-tracted	Ex-tracted with ethyl acetate	Ex-tracted with benzene	Unex-tracted*	Ex-tracted with ethyl acetate	Ex-tracted with benzene
1. Mrs. A. P. 7 mos. pregnant.....	5100	3100			15000	
2. Mrs. P. 7 mos. pregnant.....	4000	1800	2800		14000	16000
3. Mrs. L. 6 mos. pregnant.....	1200	620	950		3300	5000
4. Mrs. C. 6 mos. pregnant.....	2500	770	2400		20000	30000
5. D. L. (a) 6 mos. pregnant.....	3000		8600	15000		20000
			6000†			15000†
(b) 7 mos. pregnant.....	3100		15000	42000		44000
(c) 8½ mos. pregnant.....	2900			115000		
6. L. L. (a) 6 mos. pregnant.....	1500		750	3000		3700
						3200†
(b) 7 mos. pregnant.....	970			24000		
(c) 8½ mos. pregnant.....	42000			95000		
7. M. M. (a) 6 mos. pregnant.....	600		470	4400		4500
			440†			3900†
(b) 8 mos. pregnant.....	35000			53000		
8. E. G. (a) 5½ mos. pregnant.....	810		810	3500		4900
			580†			3600†
(b) 7 mos. pregnant.....	3200			55000		
9. Mrs. S. 3 mos. pregnant.....	180		520	780		900
			390†			750†
10. Mrs. D. G. 4 mos. pregnant.....	1060			5300		
11. R. M. (a) 6 mos. pregnant.....	1500			37000		
(b) 7 mos. pregnant.....	11000			55000		

* Small amounts of these urines were acidified, boiled and diluted for assay so that the final concentration of HCl was not more than 1.5 per cent. No appreciable loss in potency, however, followed adjustment of the pH to 7-4, provided the specimens were assayed immediately.

† For comparison these extracts were taken up in normal saline solution instead of olive oil.

TABLE 1—*Concluded*

SOURCE OF SPECIMEN	OESTRIN RECOVERY—RAT UNITS IN 24° VOLUME					
	Untreated (pH of 7.0 to 4.0) "Free"			Boiled with 15 per cent HCl—"Total"		
	Unex-tracted	Ex-tracted with ethyl acetate	Ex-tracted with benzene	Unex-tracted*	Ex-tracted with ethyl acetate	Ex-tracted with benzene
12. Mrs. S. D. (a) 5 mos. pregnant..	1100			5500		
(b) 6 mos. pregnant.	3000			24000		
(c) 6½ mos. pregnant.	3100			37000		
(d) 8 mos. pregnant.	16000			38000		
13. Mrs. G. 3½ mos. pregnant.	1000			3500		
14. L. S. 4½ mos. pregnant.	780			4600		
15. Mrs. M. W. 7 mos. pregnant. ...	3400			20400		

concentrated hydrochloric acid, 5 to 10 minutes with 15 per cent being sufficient.²

If the augmented oestrogenic potency of pregnancy urines after boiling with acid were the result of a transformation of the hormones present into more active structural forms (7), one would expect a constant percentage increase to follow this treatment. The results in table 1 show that this is not the case. In order to be certain of this point, however, the effect of acid treatment was tried upon a pure compound. For this purpose theelin (Parke, Davis & Co.), the crystalline preparation of ketohydroxyoestrin, was used. Three different lots, all over a year old, were tested. When first diluted for injection they were found to contain 40, 30 and 30 r.u. per cc. respectively. When boiled for 10 minutes with 15 per cent hydrochloric acid, they titrated at 75, 60 and 50 r.u. per cc. respectively. However, when the untreated, diluted solutions were allowed to stand for 10 days or more before testing, they also contained 75, 60 and 50 r.u. per cc. respectively. Needless to say, this unexpected finding was checked enough times to convince us of its verity. The only apparent explanation is that by long standing in the slightly alkaline medium in which it is

² Cohen and Marrian (11), in an article which appeared when this work was practically completed, report briefly their experiments on acid treatment of the urine of pregnant women. They acidified with HCl to a pH of 1 to 2 and found that in order to get the greatest oestrogenic potency without any destruction, it was necessary to autoclave at 15 pounds' pressure for 2 to 4 hours. It seems probable that our method, using a great deal higher concentration of acid and heating for a much shorter time, has the same final effect.

TABLE 2

Effect of varying the amount of acid or the time of boiling upon the yield of "total" oestrin from a pregnancy urine

Each sample represents 6 cc. of the specimen plus acid, heated, and then diluted to 200 cc. for assay.

SAMPLE NO.	HCl ADDED BEFORE HEATING	TIME OF ACTUAL BOILING	TIME IN STEAM BATH AT 100°C.	ACID CONCENTRATION AT TIME OF ASSAY	YIELD OF OESTRIN r.u./24°
	<i>per cent</i>				
1	15	5 minutes		pH 7-4*	78000
2	15	5 minutes		0.45 per cent	78000
3	7.5	5 minutes		pH 7-4*	16000
4	30	5 minutes		pH 7-4*	78000
5	15	1 minute		pH 7-4*	11000
6	15		10 minutes	pH 7-4*	39000
7	15		20 minutes	pH 7-4*	52000
8	15		1 hour	pH 7-4*	78000
9	15	1 hour with re- flux condenser		0.45 per cent	78000
10	15		2 hours	pH 7-4*	62000
11	15		10 hours	0.45 per cent	39000

* Neutralized just before assay.

TABLE 3

Values for "free" and "total" oestrin in the urines of a woman during the third month of pregnancy; demonstrating the consistent yields of "total" oestrin, and the quantitative inadequacy of assays on untreated specimens

	URINARY OESTRIN—RAT UNITS IN 24 HOURS		
	"Free" Fresh concentrated urines of pH 7 to 4		"Total" Boiled 5 minutes with 15 per cent HCl- benzene extract
	Unextracted	Benzene extract	
2 months pregnant	Less than 45	14	73
7th day of 3rd month	130	37	270
9th day of 3rd month	Less than 66	28	250
11th day of 3rd month	Less than 50	11	320
13th day of 3rd month	58	35	350
15th day of 3rd month	Less than 72	300	340
17th day of 3rd month	150	91	320
19th day of 3rd month	115	130	370
21st day of 3rd month	Less than 70	220	515
23rd day of 3rd month	174	325	650
25th day of 3rd month	280	540	630
28th day of 3rd month	178	380	950
3 months pregnant	185	520	1000
4th day of 4th month	180	190	1340

made up theelin was bound, and that merely by standing after dilution its potency was regained. Acid treatment, therefore, merely immediately broke up whatever combination it was that rendered the original hydroxy-ketone partially inactive.³ It seems probable that some analogous process occurs in urines. We were unable to find any relation between the pH of urine specimens and the "free" oestrin, but variations in other urinary constituents (depending possibly upon the diet of the individual) might well determine the amount of oestrin demonstrable in untreated urines. We assume for the present, then, that boiling urine with acid does not produce a structural change in the hormone with resultant greater activity,

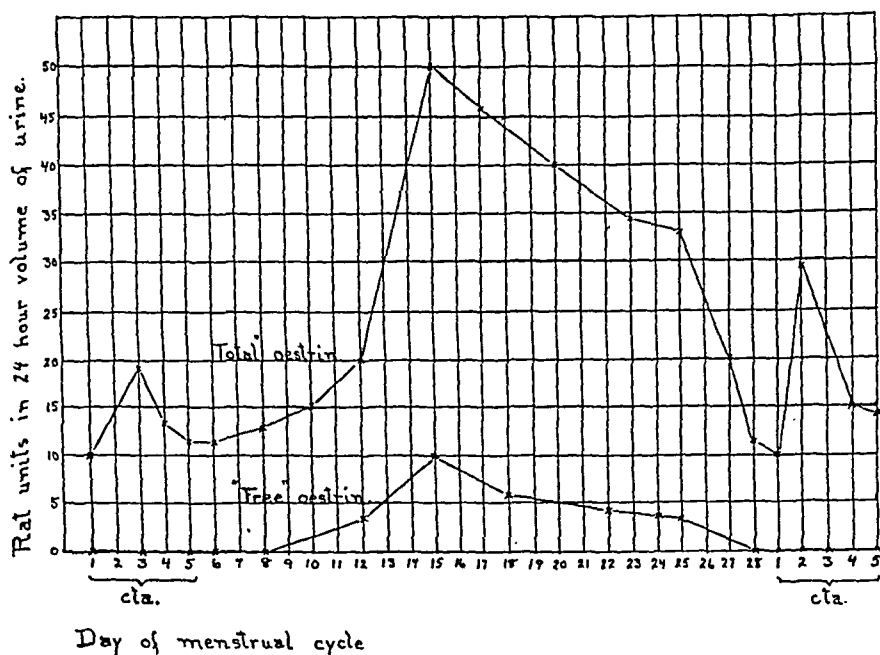


Fig. 2. Demonstrating the difference between "free" and "total" oestrin (ethyl acetate extraction) in the urines of a normal woman throughout one menstrual cycle.

but simply "frees" (possibly through hydrolysis) oestrin which was already present but bound by urinary constituents.

Table 3 strikingly illustrates the importance of acid treatment in the quantitative analysis for urinary oestrin. The urines were all extracted and tested while fresh. They were concentrated specimens and no adjustment of pH was necessary. The values for "total" oestrin follow a uniform curve, rising steadily as pregnancy advanced. The values for "free" oestrin, however, are inconsistent from day to day.

³ These results have been confirmed by similar experiments with theelol, the crystalline preparation of trihydroxyoestrin (Parke, Davis & Co.), which is also made up in a slightly alkaline solution.

The curves in figure 2 illustrate the difference between "free" and "total" oestrin in the urines of a woman throughout a normal menstrual cycle. Although the values on both treated and untreated specimens show a peak at the 15th day, the curves are only roughly parallel. The ratios vary between 1 to 5 and 0 to 30. We as yet do not know whether or not this is a typical curve. It is presented merely to illustrate the inadequacy of tests on untreated urines and cannot be compared with curves which have been published by others. These analyses were performed before we had adopted the benzene extraction, so that with this better method higher values for "total" oestrin and a greater degree of accuracy may be expected.

In table 4 are the analyses of 14 urines from 12 non-pregnant individuals,

TABLE 4

Comparison of "free" with "total" oestrin in the urines of non-pregnant individuals

NAME	MODE OF EXTRACTION	URINARY OESTRIN—r.u. IN 24° VOLUME	
		"Free" pH of 7 to 4	"Total" Boiled 5 minutes with 15 per cent HCl
1. Mrs. S.	Ethyl acetate	6	60
2. Rosalie S.	Ethyl acetate	6	10
3. Miss V. P.	Ethyl acetate	Negative for 2.5 r.u.	10
4. Mrs. G. H.	Ethyl acetate	2.5	95+
5. Mrs. G. G.	Ethyl acetate	Negative for 2.5 r.u.	Negative for 10 r.u.
6. Mrs. M.	Benzene	20	30
7. Mrs. A. M.	Benzene	20	20
Same patient one week later.	Benzene	Negative for 2.5 r.u.	25
8. Mr. R. W.	Benzene	Negative for 2.5 r.u.	15
9. Mr. R. W.	Benzene	75	400
10. Mrs. S.	Benzene	Negative for 2.5 r.u.	10
11. Miss H.	Benzene	60	60
12. Mrs. A. G.	Benzene	20	100
13. Rita S.	Benzene	Negative for 2.5 r.u.	10

both before and after acid treatment. Here again the inconsistency of the ratios of "free" to "total" oestrin are demonstrated. Case histories were purposely omitted, since no clinical significance may be attached even to the results for "total" oestrin until the limits for normal excretion have been established.

The experiments outlined indicate that the amount of oestrin demonstrable in untreated urines depends upon variable interfering substances and not upon the physiological condition of the patient. When urines are tested for "total" oestrin only, the whole specimen may be boiled with acid and extracted. In only one instance (no. 5, table 4), have we found less than 10 rat units of "total" oestrin in the urines of either normal or

abnormal non-pregnant individuals, so that at least 10 animals may be used for assay. Since most specimens contain considerably more oestrin than this, it is hardly ever necessary to extract the whole 24-hour amount.

The final procedure that we have adopted in the analysis of urines from non-pregnant individuals is as follows: A 24-hour volume is collected and the volume measured. We are in the habit of making creatinine determinations upon all specimens. Together with the weight of the patient, this gives a fairly accurate gauge as to whether or not a true 24-hour volume has been collected. For each liter of a 24-hour volume of non-pregnancy urine, 200 to 800 cc., depending upon the amount of oestrin probably present, are measured into an Erlenmeyer flask. Fifteen volumes per cent of concentrated hydrochloric acid are added and the mixture heated to boiling and boiled vigorously for 10 minutes. The material is then transferred to the large extraction flask (see fig. 1). This flask and the smaller one are both filled to the neck with benzene, the apparatus connected and the extraction carried on for 24 hours. At the end of this time the large flask, containing urine and benzene, is disconnected, emptied, put back in place and most of the benzene in the smaller flask distilled over into it. The benzene so recovered contains no oestrin and may be used repeatedly. In the case of large specimens containing small amounts of oestrin, two runs must be made, since the capacity of the apparatus is 1000 cc. The benzene extracts are then combined and transferred to a small beaker, washing with benzene. Six cubic centimeters of olive oil are added and the benzene evaporated off.

In assaying we usually test first with 0.75 cc. of olive oil extract upon each of 2 animals; i.e., for 10 to 40 r.u. of "total" oestrin per 24 hours, depending upon the amount of urine extracted. If negative, progressively larger, and if positive, progressively smaller, amounts of extract are tested upon 2 or more rats at a time until the minimum dose that will produce oestrus in half the animals is discovered. The more oestrin present, therefore, the more rats used, and the more accurate the test. Often the extracts must be diluted with olive oil for the final assays.

Calculation:

$$\frac{(24^{\circ} \text{ volume} \div \text{cc. extracted}) \times 6}{R} = \text{r.u. in } 24^{\circ} \text{ volume}$$

where R stands for the smallest amount of extract that will produce oestrus.

SUMMARY AND CONCLUSIONS

A series of comparative analyses demonstrated that neither chloroform, olive oil, ethyl acetate nor benzene could be counted upon to give complete recovery or quantitatively comparable percentages of oestrin as it occurs

in untreated pregnancy urines. Chloroform gave the lowest and benzene the highest values.

Recovery experiments, employing 24-hour continuous extraction with benzene, revealed that assays on untreated pregnancy urines could not be used as a gauge of the amount of oestrin actually present, since in some cases more was recovered than had been demonstrable in the unextracted specimens.

Pregnancy urines, after being boiled for 5 to 10 minutes with 15 volumes per cent of concentrated hydrochloric acid, increased greatly in their oestrin content. There was, however, no constant ratio between the amount of oestrin found in the untreated specimens and the amount "freed" by acid treatment. The ratios varied between 1:1.5 and 1:40.

Benzene extraction was shown to give as quantitative a recovery of the oestrin "freed" by acid treatment as could be expected when using a biological means of assay. With 3 urines ethyl acetate extracted 12 to 34 per cent less "total" oestrin than did benzene.

Experiments in which the concentration of acid and the time of boiling were varied demonstrated that no greater increase in potency could be produced by changing the technique within certain limits, and that no destruction of "freed" hormone resulted from boiling for 10 minutes with 15 per cent acid. This procedure, therefore, was adopted and the oestrin so recovered called "total."

In assays upon the pure hydroxyketone, theelin, it appeared that upon long standing in the slightly alkaline solution in which it is put up, theelin was bound, since when first diluted for injection the potency was considerably less than could be demonstrated after the diluted solution had stood for 10 days or more. Acid treatment did not result in any further increase in its oestrogenic activity. It was concluded, therefore, that acid treatment did not produce a more potent oestrogenic form but simply "freed" the theelin which was originally present. No relation between the pH of urine specimens and the amount of "free" oestrin could be established, but it seems probable that some analogous process occurs and that the amount of oestrin "free" at any one time in any one specimen depends upon variations in the other urinary constituents.

From repeated analyses over a period of one month upon the fresh, slightly acid urines of a pregnant woman, it was shown that, whereas the values for "free" oestrin were strikingly inconsistent from day to day, the "total" oestrin followed a uniform curve, rising steadily as the pregnancy advanced.

"Free" and "total" oestrin were extracted from the urines of a woman throughout a normal cycle. The curves ran roughly parallel, but the actual ratios of "free" to "total" varied between 1:5 and 0:30.

The results on single specimens from 12 non-pregnant individuals showed ratios of "free" to "total" oestrin varying from 1:1 to 1:35+.

An outline of the exact procedure finally adopted for the assay of oestrin in urines from non-pregnant individuals is given.

It is concluded that this procedure gives physiologically significant values, whereas the usual methods of analyses upon untreated urines may result in completely misleading figures bearing no relation to the amount actually present.

It at first seemed possible that the increase in oestrogenic potency of urines after boiling with acid might be due to the transformation of some physiologically inactive oestrin precursor (e. g., possibly pregnandiol) into an active form. The results tabulated, however, especially in tables 1 and 3, are inconsistent with this interpretation, and indicate that the physiologically significant values are represented by the figures for "total" oestrin only.

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THE EFFECT OF CORONARY OCCLUSION ON MYOCARDIAL CONTRACTION¹

ROBERT TENNANT² AND CARL J. WIGGERS

*From the Department of Physiology, Western Reserve University Medical School,
Cleveland, O.*

Received for publication March 22, 1935

Many students of the coronary circulation must have noted that the ventricular zone affected by ligating a large coronary branch not only appears cyanotic and dilated, but that it seems to alter in its mode of contraction. The detailed and sequential changes in contraction are not easily followed by the unaided eye and so far have not been recorded myographically. The reasons for this were the lack of an adequate and suitable myograph and a technic for the application of one to a limited ventricular surface so that records obtained represent, at least reasonably well, changes in muscle length and not predominantly artefacts due to position changes, thrusts and vibrations of the vigorously beating ventricle.

This communication concerns itself with descriptions of a technique and of a type of optical myograph suitable for such studies and an analysis of the changes in optical myograms which follow clamping of a large coronary vessel.

APPARATUS. After preliminary efforts to obtain satisfactory ventricular myograms with the segment myograph used by one of us (Wiggers, 1916) to study auricular contraction, it became obvious that in order to overcome the distortions produced by twists and thrusts of the beating ventricle an instrument was needed in which the movable lever arm operates in fixed bearings. A suitable myograph which retains the compactness, lightness and efficiency of the earlier form is illustrated in figure 1. The body of the instrument consists of a small receiving tambour, *E*, (2.5 cm. in diameter) from which a tube leads off at right angles for connection with an optical segment capsule. The lever arms which are of aluminum are spaced 1.5 cm. apart. The rigid arm, *A*, is attached solidly to the back of the tambour and the movable one, *B*, is pivoted in jewel bearings, *C*, as indicated in the insert sketch. The total weight of the myograph is only

¹ The expenses of this investigation were defrayed partly from a grant to one of us (C. J. W.) by the Ella Sachs Plotz Foundation, and partly from a grant to the other (R. T.) by the Josiah Macy, Jr. Foundation.

² Fellow of the National Research Council.

10 grams, hence its attachment does not modify cardiac contraction. When the lever arms are securely stitched through the eyelets to the ventricular surface their approximation compresses the tambour rubber, *D*. The pressure changes thus created are transmitted to a Frank capsule optically so arranged that the recorded curve is upward in direction. The

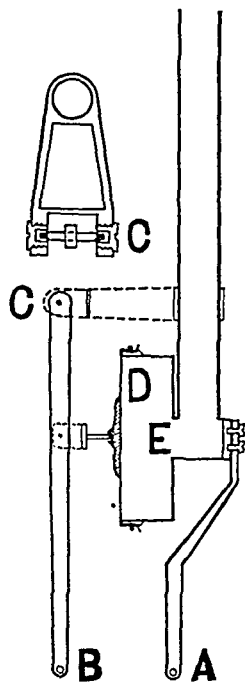


Fig. 1. Diagram illustrating ventricular myograph for use with optical capsule. *A*, fixed lever arm; *B*, movable lever arm, mounted in jewel bearings at *C*. *D*, rubber dam covering small tambour, *E*, with right angle lead-off tube. (Actual size.)

myograph arms must be firmly attached to the ventricular surface and exactly aligned in the direction of the superficial muscle fasciculi of the region studied. Twisting action still exerted on the myograph by the contractions of other fibers can generally be eliminated if the rubber tube connection on the lead-off tube is turned in one direction or the other until such motions are gone. To minimize the transference of finer vibrations, such as heart sounds, the tambour is covered with a relatively thick rubber dam (0.29 mm.) and the Frank capsule with a dam as heavy as the registration of curves of proper amplitude permits.

The attached myograph is suspended by an elastic band, the tension of which must be meticulously adjusted so that a light pull is exerted upon the stitches and underlying myocardium. With proper adjustment of the tension the up and down movements of the heart as a whole are not recorded. If the size of the ventricle changes, readjustments of tension can be made conveniently by means of a screw control at the upper end of the elastic suspension.

In addition the exposed heart is so adjusted in a pericardial cradle that the region selected for study moves as little as possible. The central anterior surfaces of both the left and right ventricles are obviously most suitable for recording good myograms, although even the extreme apex and basal regions with their extensive movements yield satisfactory records.

METHODS. Dogs 10 to 20 kilos in weight were anesthetized with morphine and sodium barbital and under mild artificial respiration the heart was exposed and rested in a pericardial cradle. In most experiments the *ramus descendens anterior* of the left coronary artery was isolated within 2 cm. of its origin in preparation for the application of a miniature clamp, and the myograph was stitched to the central anterior surface of the left ventricle. In a few experiments the right coronary artery

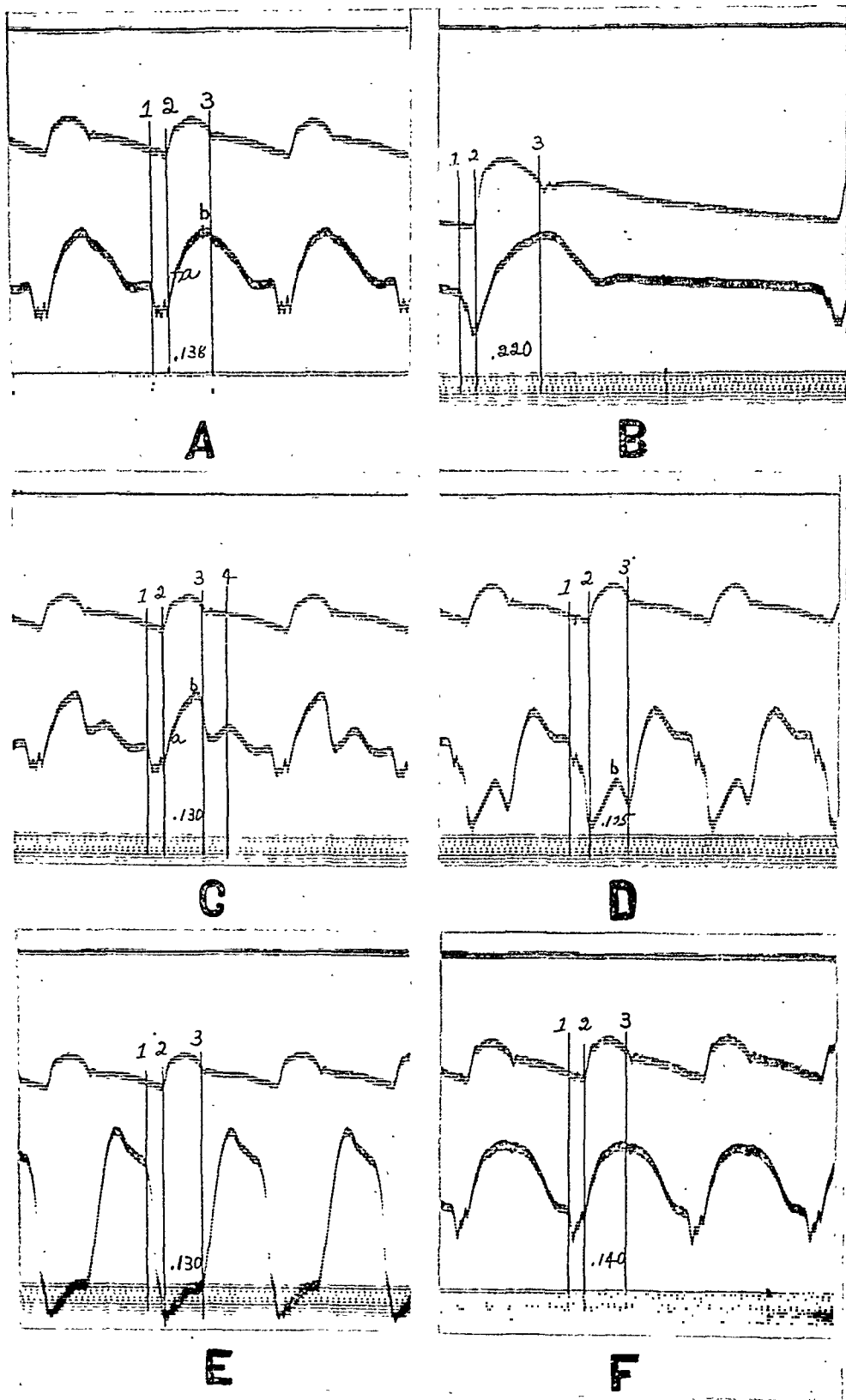


Fig. 2. Six segments from records showing A, control B, slow beat, C, D, E, evolving changes in left ventricular myogram during left coronary occlusion and F, recovery following release. Upper curve aortic pressure, lower, myograms. Time, 0.02 second. Further discussion in text. (Reduced.)

was isolated instead and the myograph similarly applied to the right anterior ventricular surface. In some experiments a second myograph was attached to a region of ventricle not supplied by the coronary vessel to be occluded.

In connection with such optical myograms, pressure pulses from either the aorta or the left ventricle were recorded in the usual manner by means of calibrated optical manometers. After satisfactory control records had been obtained the isolated coronary vessel was securely clamped and synchronous optical records were taken either continuously on slowly moving paper or at frequent intervals after occlusion on rapidly moving paper. In a similar fashion the effects of decompression of the coronary artery were also studied after varying intervals of occlusion. During

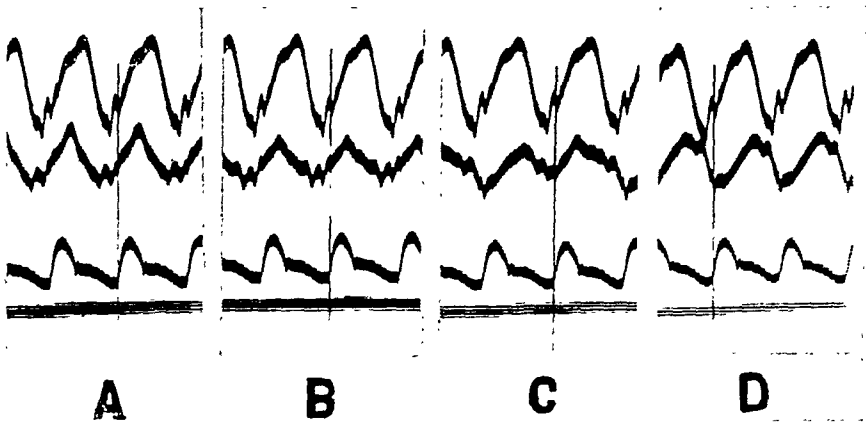


Fig. 3. Four segments of records showing A, control B, C, D, evolving changes of right ventricular myogram (middle) and unaltered character of left ventricular myogram (upper) following ligation of right coronary artery. Lower curve, aortic pressure. (Reduced.)

several experiments, ventricular fibrillation occurred either before or just after the release of the clamp. In a large proportion of such accidents fibrillation was abrogated and a perfectly normal beat reestablished by applying electrodes to the heart and sending brief counter-shocks of an A-C current directly through it, as described by Hooker, Kouwenhoven and Langworthy (1933).

Characteristics of the normal ventricular myogram. Myograms recorded from different accessible regions of the right and left ventricle are essentially alike. Typical tracings from the left ventricle are shown in figure 2, A and B, in which curves with rapid and slow rates of beat are illustrated. Other examples are shown in figure 3 (upper curve) and in curve A of figure 4.

During the isometric contraction phase (indicated by lines 1-2) the

myograms show two characteristic deformations, i.e., either a steep drop followed by several small oscillations or a transient sharp positive spike. Frequently less conspicuous oscillations or dips characterize this phase. All of these must be considered as artefacts which we have not succeeded in eliminating.

Precisely with the onset of ejection (line 2) the myogram at first rises steeply (2-a) and then more gradually to a summit near or at the end of systole (line 3). This portion of the curve corresponds to the auxotonic shortening process. The summit generally persists during isometric relaxation (fig. 2, B and F) or sometimes a further small elevation follows the incisura which is an artefact (fig. 4, A). With the onset of ventricular filling the curve declines rather rapidly to a basic diastolic level. In cycles with a long diastole (fig. 2, B) a slight gradual after-stretching occurs during diastasis.

Changes in myograms following occlusion of ramus descendens anterior. Continuous records taken after occlusion show a series of evolving changes in the contour of the contraction curve leading in about a minute to its complete inversion during systolic ejection (fig. 2, E). The detailed evolution is shown in curves of figure 4. Although the myograms alter from beat to beat they may for convenience be described as of three categories, viz., 1, an initial type characterized by smaller amplitude and decreased duration of contraction (fig. 2, C; fig. 4, B-F); 2, transitional types (fig. 2, D; fig. 4, G, H, I) indicating progressive decrease in shortening and a struggle between the forces causing shortening and those tending to lengthen the fibers, and 3, frankly inverted myograms (fig. 2, E; fig. 4, J-K). A detailed study of myograms recorded immediately following occlusion shows in addition to a decreasing amplitude of contraction a concomitant shortening of the period of contraction of the affected muscle (2-b) as illustrated in A, C and D of figure 2. The duration of systolic ejection also decreases during this interval and usually remains reduced until some time after frank inversion of the myogram has occurred when it may again come to equal the initial duration.

Analysis of the inverted myogram (fig. 2, E; fig. 4, J-K) brings out several significant changes in time relations. The chief abrupt drop of the curve,

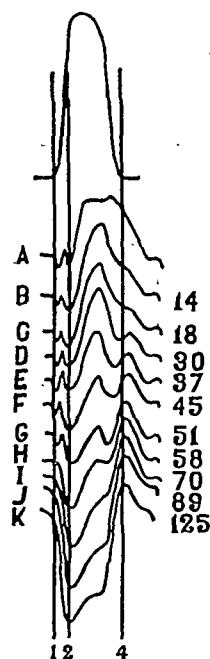


Fig. 4. Series of transcribed left ventricular myograms from a continuous record (A-K) showing sequential changes following ligation of left ramus descendens, in relation to a left ventricular pressure curve (upper). Numbers to right denote number of beats following occlusion. Discussion in text.

indicating expansion of the ischemic muscle, occurs during the isometric rise of intraventricular pressure and prior to the rise of aortic pressure. During systolic ejection (2-3) a slight degree of muscle shortening generally occurs; the curve is rarely an inverted image of the intraventricular pressure summit. Precisely synchronous with the incisura of the aortic pressure curve (3) and the rapid fall of intraventricular pressure during isometric relaxation the curve rises sharply to the diastolic level with a subsequent gradual decline as the ventricle fills during diastole.

TABLE 1

EXP. NO.	DURATION OF LIGATION	TIME INTERVAL BETWEEN LIGATION AND FAILURE	RECOVERY	TIME INTERVAL BETWEEN RELEASE AND RECOVERY	REMARKS
1	1 min. 40 sec.	50 sec.	Yes	45 sec.	Without fibrillation
2	2 min. 20 sec.	50 sec.	Yes	30 sec.	Without fibrillation
3	2 min. 30 sec.	35 sec.	No		Fibrillation without revival
4	2 min. 30 sec.	20 sec.	No		Fibrillation without revival
5	3 min.	75 sec.	No		Fibrillation without revival
6	3 min.	150 sec.	No		Fibrillation without revival
7	4 min.	55 sec.	Yes	11 min.	Fibrillation revived
8	4 min. 44 sec.	90 sec.	Yes	1 min. 40 sec.	Without fibrillation
9	4 min. 45 sec.	70 sec.	Yes	14 min.	Fibrillation revived
10	10 min. 30 sec.	40 sec.	Yes	5 min. 30 sec.	Fibrillation revived
11	23 min.	65 sec.	Partial	8 min.	Fibrillation revived
12	45 min.	150 sec.	No		Heart beat for 2 hours after release
13	46 min.	80 sec.	No		Fibrillation without revival
14	1 hr. 50 min.	31 sec.	No		Fibrillation 14 minutes after release and revived for 8 minutes

These changes persist until the ventricle fibrillates or stops in a hypodynamic state as is usual in most acute experimental occlusions. In some instances, however, as in the series reported by Orias (1932) an effective ventricular action continued for 1 to 1½ hour despite the constant lack of any significant contraction in the ischemic zone.

When in such experiments the coronary clamp is released after a short period of closure, a reversed set of evolving changes occurs leading rapidly to a complete restoration of normal contractions in the zone (fig. 2-F).

A determination of the maximal duration of ventricular ischemia compatible with such recovery requires a much larger series of observations than are at our disposal at present, although the data of table 1 indicate that after intervals of 23 minutes or greater, recovery was only partial or absent even an hour later.

The excitability and conductivity of the ischemic area. The absence of contraction in the ischemic zone may be due either to a depression of the contractility of the affected muscle or to a failure of impulses to reach it because of a loss of conductivity and/or irritability. That these latter functions are not essentially disturbed was demonstrated by the following experiments:

Myographic records were simultaneously recorded from the right and left ventricles. The anterior descending ramus of the left coronary was ligated and after several minutes when the myogram of the left ventricle showed distinct inversion the heart was stopped by vagal stimulation and the ischemic zone stimulated with rhythmic break induction shocks of a strength which had previously been found just adequate to excite the normal ventricle. With this stimulation impulses spread in the usual manner to the right and left ventricles and caused their contraction, although each elevation of left intraventricular pressure continued to expand the ischemic area. This demonstrates that the functions of contractility can be seriously impaired by anoxia while the properties of irritability and conductivity are essentially unchanged.

The ineffectiveness of perfusions with Locke's or Tyrode's solutions. In an effort to evaluate the minimal oxygen supply capable of sustaining muscular contraction it was proposed to perfuse the descending ramus of the left coronary artery with fully oxygenated Locke's or Tyrode's solutions and then by progressive gradual decreases in oxygen pressures and simultaneous time flow measurements to calculate the minimal oxygen requirement necessary to sustain contraction. Since no difficulty was encountered by us in maintaining beats in the hearts of small dogs perfused by the Langendorff method with the same solutions and since various workers in this laboratory had shown that fibrillation and hypodynamic failure so common in dogs after coronary ligation could be prevented by such perfusion even at low pressures the plan seemed perfectly feasible. It immediately became apparent, however, that even at the highest oxygen and perfusion pressure compatible with experimental methods (200 mm. Hg above atmospheric pressure) such solutions are certainly incapable of maintaining sufficient contraction in the perfused zone to cause a shortening with the heart performing work under natural conditions. The myographic curves from these zones invariably resembled those of figure 2, E. Since it can be shown by simple calculations that such solutions even under these high O₂ tensions cannot absorb more than 2.5 volumes per cent of

oxygen, coronary flow cannot be made great enough to supply sufficient oxygen for supporting efficient contractions in the normally working heart. Further studies employing pure hemoglobin solutions will be required to determine the minimal oxygen requirements.

The responses of the right ventricle during ischemia. Whether the contractile failure will eventually prove to result from exhaustion of and a failure to resynthesize phosphocreatine, from accumulation of lactic acid, from decrease in pH, or less probably from failure in oxidation of lactic acid there can be no doubt but that anoxia due to an inadequacy of collateral blood supply is the tangible factor. Inasmuch as the Thebesian and other communications are anatomically more extensive in the right heart and since its total metabolic requirements are less, the question arises whether a similar prompt reduction and failure of efficient contraction occurs after occlusion of the right coronary artery.

To study this question the right coronary artery was isolated and compressed and the effects on myograms recorded from both right and left ventricles were compared. Such experiments illustrated by 4 segments of records in figure 3 demonstrated that changes identical with those described in the case of the left ventricle are certain to follow occlusion of the right coronary artery, although the time required for complete reversal was regularly somewhat longer (up to 3 or 5 minutes). In these records the upper curves are myograms from the left ventricle, the middle curves, myograms from the right ventricle, and the lower, an aortic pressure curve. The right myograms in segment B, C and D show respectively the initial depressed, the transitional and the frankly inverted characteristics, while the character of the left ventricular myogram remains unaltered. Apparently, the advantages gained by the better collateral communications of the right coronary system with the ventricle are largely offset by the fact that they necessarily carry less completely oxygenated blood than do the main vessels.

DISCUSSION. The interesting and somewhat surprising discovery that approximately one minute after coronary occlusion the contractile force in an ischemic area is either abrogated or certainly so feeble that the ischemic muscle stretches instead of shortens during systole and in proportion to the elevation of ventricular pressure, has many implications of importance to clinical medicine and experimental physiology. Of these we shall discuss a few:

1. Our results demonstrate more convincingly than direct circulatory studies the functional inadequacy of anatomically described collateral branches to ventricular muscle. Furthermore, the numerous instances of infarction and cicatrization found postmortem after coronary occlusion in humans and in dogs (experimentally produced) indicate that circulatory conditions are not dissimilar in these hearts. Consequently our observa-

tions strongly suggest that if an extensive collateral circulation has not developed prior to a total occlusion, the muscle in the zone affected is not likely to survive.³

2. Since the muscle fibers in the affected zone promptly undergo periodic stretching instead of shortening, the thought arises that such mechanical factors, rather than chemical, as commonly postulated, may be the ultimate stimulus to the sensory nerves and so account for the immediate intense pain associated with the occlusion. Upon such an assumption, the hitherto unexplained benefits of pressure lowering drugs would be clarified since, by lowering the maximum intraventricular pressure, the degree of the periodic stretching would be reduced. This possibility is worthy of more extensive investigation.

3. Experimentally our observations supply tangible proof for certain logical assumptions that Orias (1932) while working in this laboratory was obliged to make in order to explain the pressure changes immediately following coronary occlusion. To account for the immediate reduction in duration of ventricular systole, this investigator postulated a prompt reduction of contractile power in the ischemic area. In order to account further for the negligible decline of systolic pressure or in some instances its complete absence before compensatory reactions of other regions had had time to develop required the further assumption that the contractions in the ischemic area were of shorter duration. Our observations have demonstrated the occurrence of both conditions. Myographic curves have shown that localized anoxemia produces the same abbreviation of contraction in the affected zone that generalized anoxemia does upon the whole heart (Sands and DeGraff, 1925). No evidence was obtained however that it exerts an initial increase in contraction as seemed to follow from studies of general anoxemia.

Our observations that a marked systolic expansion of the ischemic region replaces shortening makes it evident that a considerable fraction of the total pressure developed is lost in producing such distention. We would therefore supplement Orias' theoretical analysis of fundamental mechanisms by adding the suggestion that the hypodynamic levels which so often occur despite a rise of initial tension may not necessarily be due to a fatigue of the remaining contraction fractions, but can be accounted for by the loss of pressure in expanding the regions in which contractions are enfeebled or absent.

4. The myographic study of localized ventricular areas which we have

³ Further experimental support for this conception has been obtained since this paper went to press. Inasmuch as a free collateral flow might conceivably be prevented by occlusion, the peripheral end was incised so that a free flow of any blood from collateral sources could occur. This was found to be without effect either in preventing or delaying the loss of contractile power.

introduced and a knowledge of the sequential changes that follow deprivation of blood supply should prove useful in estimating the value of drugs in coronary occlusion, both those that might insure a better collateral supply and those that might act through a direct effect on the muscle. Unless it can be shown that some degree of immediate improvement in contraction occurs in the regions affected, no great practical value as regards maintenance of function and avoidance of subsequent pathological changes can be anticipated.

SUMMARY

1. An optical myograph suitable for recording localized contractions from a ventricular surface and a technique for its correct application are described.

2. Normal myograms recorded simultaneously with aortic or ventricular pressure curves, though slightly deformed by oscillations during the isometric contraction and relaxation phases clearly show the natural shortening which occurs during ventricular ejection and the lengthening which follows isometric relaxation.

3. Occlusion of a main coronary branch is followed by an evolving series of myographic changes which indicate progressive enfeeblement of contraction to the extent that approximately within a minute the area stretches during isometric contraction, remains stretched during systolic ejection and shortens quickly during isometric relaxation; in short, the myogram is completely inverted. Similar changes in contraction of the right ventricle occur following ligation of the right coronary artery. These observations demonstrate convincingly the functional inadequacy of described collateral circulation in normal hearts.

4. Reestablishment of the normal blood supply is followed by a reversed series of myographic changes with restoration of normal vigorous contractions provided the period of ischemia is not too long in duration.

5. Failure of shortening is due to enfeeblement or abrogation of contraction and not to failure of impulses to reach the areas involved, or to excite them.

6. The oxygen requirements for maintaining efficient contractions in the normally working heart are high as evidenced by our failure to maintain efficient contractions when an area is perfusing with highly oxygenated Locke's solution.

7. The observations supply tangible proof for the correctness of Orias' hypothesis that coronary occlusion produces an early abbreviation of total ventricular systole with little or no decline of systolic pressure through a progressive decrease in amplitude and duration of contraction in the ischemic area. Our results suggest further that the tendency for development of hypodynamic ventricular beats following coronary occlusion may

not necessarily be due to fatigue of the remaining contracting fibers, but can be explained by loss of pressure in expanding the regions in which contractions are enfeebled or absent.

8. Several clinical implications of our results are briefly discussed.

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PHASIC VARIATIONS IN PERIPHERAL CORONARY RESISTANCE AND THEIR DETERMINANTS¹

DONALD E. GREGG, HAROLD D. GREEN AND CARL J. WIGGERS

From the Department of Physiology, Western Reserve University Medical School, Cleveland, O.

Received for publication March 22, 1935

Information regarding the successive changes of resistance in the coronary branches and their determinants is of paramount importance both in understanding the phasic changes of coronary flow and in interpreting the actions of drugs upon the intact coronary circulation. It seemed that a study of the pressure pulse from the peripheral end of a coronary vessel (peripheral coronary pressure—P.C.P.) should supply this information.

This report deals with a description of such pulses recorded by a calibrated optical manometer together with an analysis of their phasic variations and of their reliability in appraising the variations of peripheral coronary resistance under natural conditions.

PROCEDURE. In a first series of experiments aortic and peripheral coronary pressures were simultaneously recorded by two optical manometers (Wiggers pattern). The cannula of the coronary manometer—modified as illustrated in figure 1A—was inserted into the peripheral end of the ligated descending ramus as indicated in figure 1A, the precautions emphasized by Wiggers and Cotton (1933) being observed. Through the lateral connection and stopcock (*a*) the peripheral coronary vessel was perfused with Locke's solution, except when records were being taken. Experience had taught us that by this expedient coagulation in the cannula tip could be prevented and the danger of the heart failing through fibrillation or hypodynamic action could generally be averted.

During the course of such studies the discovery was made by Tennant and Wiggers (1935) that an area so perfused extends during systole instead of shortening. Since replacement of a normal systolic shortening by an extension might conceivably alter the peripheral coronary resistance changes, a second method for recording P.C.P. was devised by which normal contractions were retained in the area studied. The procedure consisted in isolating the anterior descendens or the circumflex ramus and also a suitable side branch and in tying the coronary cannula into the

¹ The expenses of this investigation were defrayed from a grant by the Ella Sachs Plotz Foundation.

latter, as illustrated in figure 1B. The normal blood supply to the area studied was thus kept intact, except while taking records, when the main vessel was clamped centrally to the side branch. Coagulation was prevented in the cannula by flushing it repeatedly with Locke's solution to which heparin had been added. Myographic records showed that such admixture of heparinized Locke's solution had no effect on contractions in the areas studied. By compressing the main branch for several minutes, records could also be obtained when the area supplied did not shorten. By releasing the clamp and restoring the blood supply, normal contractions returned in the area affected as described by Tennant and Wiggers (1935).

In order to improve the "figure of merit" of the manometer equipped with a relatively small cannula tip, a very tense rubber membrane was used. The resulting decrease in sensitivity was compensated for by moving the photokymograph away to a distance of 2.6 meters. Adequate

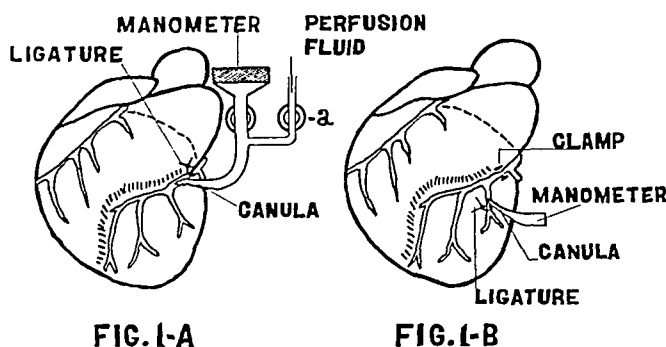


Fig. 1. Two diagrams illustrating insertions of cannulas, ligations and clampings used for studying peripheral coronary pressures.

intensity of beams was insured by using small plano-convex mirrors flooded by light from a projection bulb, as suggested by Hamilton, Brewer and Brotman (1934).

The contour of peripheral coronary pressure pulses. Typical records from a heart in which the zone determining peripheral pressure changes was presumably extending during systole (method 1) are reproduced in figure 2. The form of the aortic pressure curves and the ordinate values derived from application of a calibration-scale attest to the existence of good dynamic conditions. Record A was obtained shortly after cannulation of the peripheral coronary branch. Vertical intercepts facilitate comparison of the phasic relations of the two pressure pulses. The curves show that 0.046 second before the steep rise of aortic pressure, i.e., probably coincident with the isometric rise of intraventricular pressure, the peripheral coronary curve rises, slowly at first (A-B), then brusquely (B-C). This rise continues more gradually into the period occupied by the steep

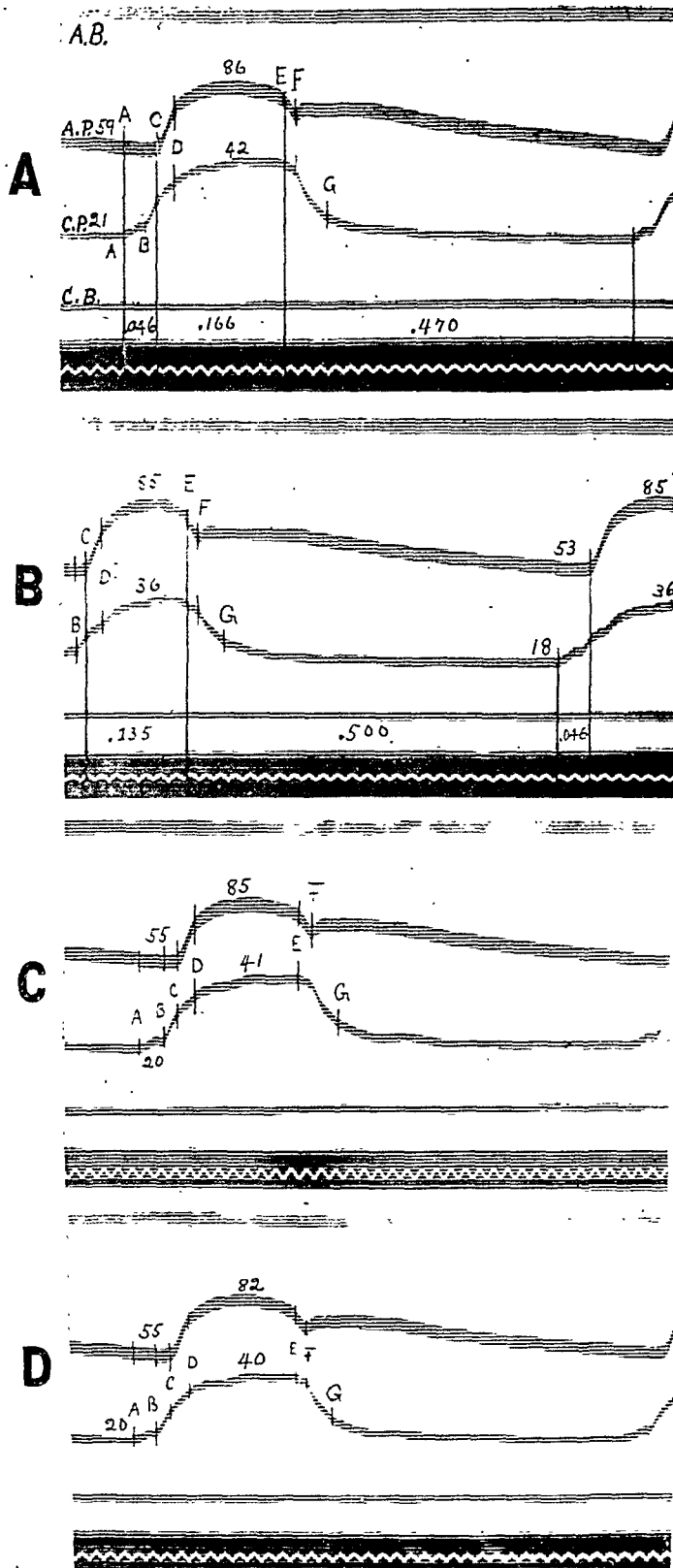


Fig. 2. Records illustrating time relations, contour and magnitude of peripheral coronary pressures. A, normal control; B, during clamping left circumflex ramus; C, after release of same; and D, during clamping of right coronary artery. A.B., aortic base line; A.P., aortic pressure pulse; C.P., peripheral coronary pressure; C.B., coronary base line. Time, 0.02 second. Discussion in text (expt. DD-1/5-8). \bar{x}

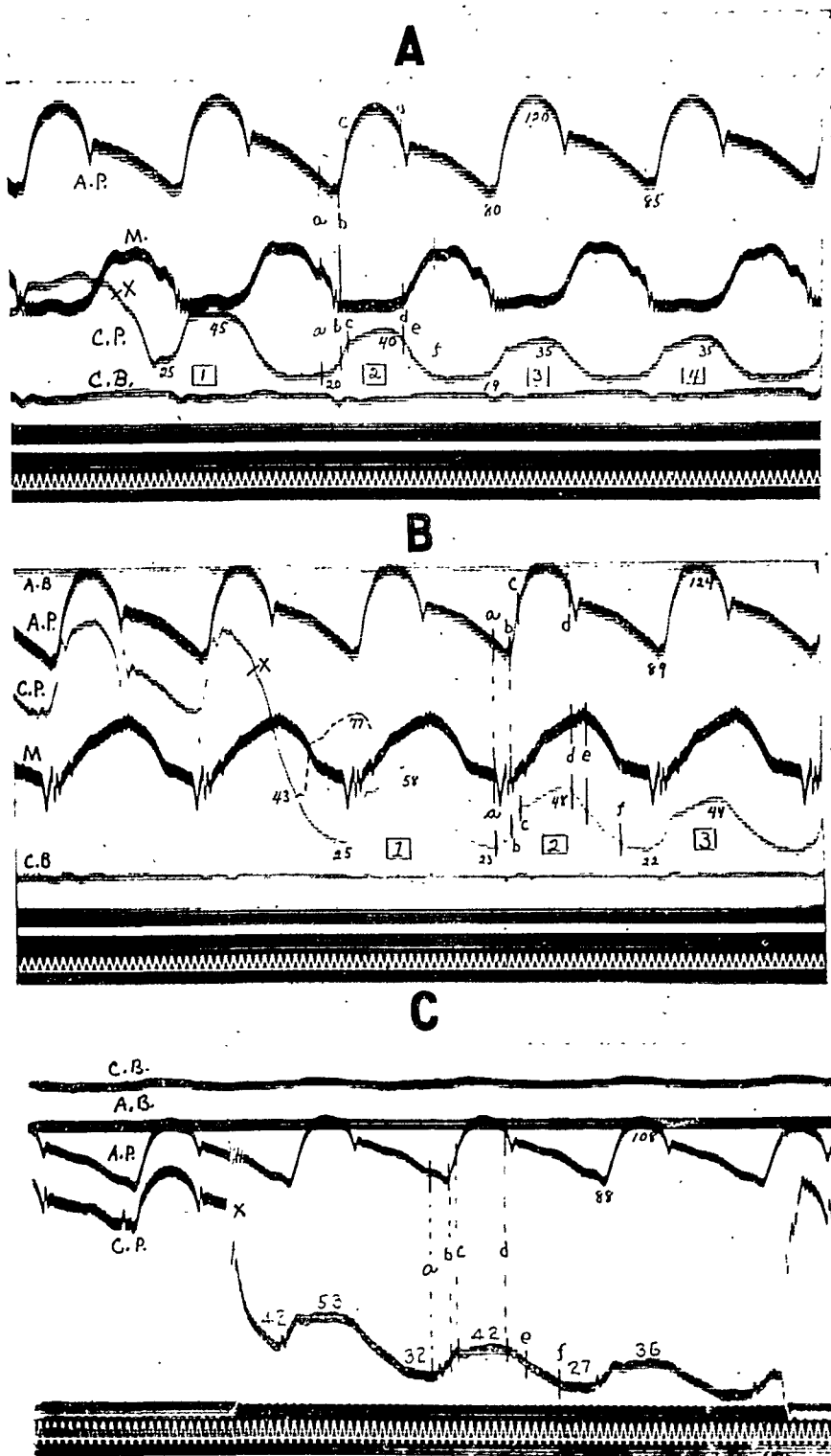


Fig. 3. Comparison of P.C.P. curves from anterior descendens ramus (A) when area extended during systole, and (B) when same area shortened. (C) record from left circumflex when area contracted. M, myogram; X, time of clamping main coronary; other lettering as in figure 2 (expt. DD-32/6-7; DD-36/10).

rise of aortic pressure (*C-D*), then mounts as a gradually rising plateau almost but not quite to the end of systole (*E*). During protodiastole (*E-F*), the curve starts to decline. At first it falls rapidly, then more slowly during the isometric relaxation phase (*F-G*). At the time ventricular inflow starts (*G*), the fall in pressure is practically completed.

The time relations and general features of the rise and fall correspond to changes of pressure within the left ventricle, but the gradually rising plateau differs essentially from the rounded summit of intraventricular pressure which may be judged from the aortic pressure summit. With minor differences these general characteristics were found in all records. In some instances the abrupt initial rise (*B-C*) extended through the period of rising aortic pressure (*C-D*). The gradient of the systolic plateau also varied in different experiments.

Examples of records in which the area was stretched (*A*) and in which it was shortening during systole (*B*), (method 2), are shown in figure 3. Record *A* was taken after the main branch of the *ramus descendens* had been clamped for 3 minutes, i.e., after sufficient time had elapsed to cause marked extension of the area supplied.² This extension is demonstrated by the myographic record which descends abruptly during isometric contraction (*a-b*) and rises with isometric relaxation (*d-f*). The peripheral coronary pressure pulses (beats 2, 3) show essentially the same features as those described above. Immediately after taking this record the coronary clamp was removed and the area allowed to recover. Record *B* in which the myogram now shows a continuous shortening during ejection (*c-d-e*) and slightly beyond it, was then recorded.

The coronary curve of this latter record starts with two beats representing lateral coronary pressure. They serve the purpose of assuring adequacy of the manometer and proper alignment between the manometer and artery. At *X* the main ramus was clamped abruptly and from this point on peripheral pressure changes were recorded.

The corresponding beats marked 2 or 3 in records *A* and *B* are very similar, the only difference noted upon close examination of record *B* being 1, a somewhat steeper rise of the plateau continuing to the very end of systole; 2, a somewhat slower gradient of decline during isometric relaxation; 3, a higher pressure maximum during systole, and 4, a trifling increase in diastolic pressure. If we accept such differences as significant it might be inferred that the existence of local contractile forces tends somewhat to augment the pressure rise during the ejection phase and to retard the decline of pressure during early diastole. In some experiments however even such differences were not apparent, while in others the initial

² Just before taking the record the vessel was distended by perfusion with Locke's solution through the side cannula and the point *X* on the record denotes the moment when it was stopped.

sharp elevation of pressure appeared to shift from the isometric period to the time that the aortic pressure rose. It should be noted however that in some experiments of our first series a steep rising plateau was present. It is significant that in all experiments the rise of coronary pressure (*a-b*) definitely preceded shortening as inscribed by the myogram at *c* and that the decline of peripheral coronary pressure definitely preceded the actual lengthening of the muscle (*e*) and began coincidentally with the incisura (*d*), i.e., with the fall of intraventricular pressure.

Record C of figure 3 is added without further description as evidence that curves similar in contour can be recorded from the peripheral end of the *circumflex ramus* (method 2). Although a myogram could not be conveniently recorded there is every reason to believe that the region affected continued to shorten, for no compression of the vessel had been made previous to this test.

The determinants of phasic variations in peripheral coronary pressure. The systolic increase in peripheral coronary pressure may be due *a*, to increased intramural or intraventricular tension; *b*, to compression of intramural vessels by shortening and thickening of muscle elements, or *c*, to transmission of pressure from collateral vessels.

In favor of the concept that *tension change* is the predominant factor are the discoveries 1, that the sharp rise and fall of peripheral coronary pressure coincide respectively with the steepest rise and fall of intraventricular pressure, but are not synchronous with the onset of shortening or lengthening recorded myographically, and 2, that as long as intraventricular pressures do not alter, the contour of the curves does not differ essentially regardless of whether the region shortens or lengthens during systole. The fact that the systolic maximum is less when the area supplied lengthens during systole (as in ischemia) can be interpreted to mean either that muscle shortening (and thickening) is normally of supplementary assistance or that stretching increases the capacity of the coronary vessels sufficiently to prevent a full development of systolic pressure (see below).

Before we may conclude that tension changes are chiefly concerned and that length changes play at most a subsidiary rôle, it is necessary to evaluate the part that pressure transmitted through collateral anastomoses with other branches may play. The magnitude of the peripheral collateral circulation in the normal heart is still unsettled, despite extensive anatomical and experimental studies. Experimental studies can easily be cited in favor of either an abundant or a negligible collateral circulation. For instance, if mean pressures are simultaneously recorded from a peripheral coronary branch, and from the aorta, the former is approximately one-fifth that in the aorta; and if aortic pressures are caused to rise by any of several methods this proportionality is roughly maintained. We have

studied such relationships extensively and have even attempted their use in evaluating the effect of drugs on the collateral supply of blood to infarcted areas. We slowly came to realize, however, that it is hazardous to conclude that such correspondence denotes a cause and effect relation, for the dynamics of the left ventricle is modified whenever aortic pressure alters (Wiggers, 1928). That increased ventricular contraction with its concordant increase of the intraventricular pressure maximum is indeed the dominant factor is shown in the record of figure 4, in which aortic and peripheral coronary pressures were recorded while the aorta was being compressed. The rise affects chiefly coronary systolic pressure; diastolic pressure is raised only to an insignificant extent. More important, however, is the fact that the higher systolic pressure occurs chiefly through a greater initial rise which precedes the elevation of aortic pressure, hence

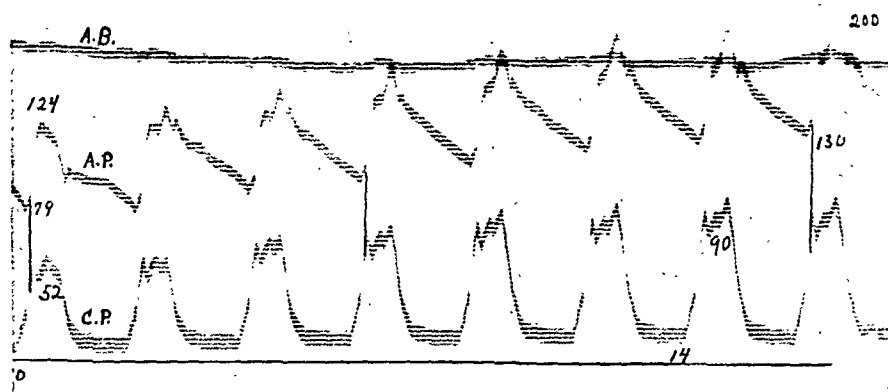


Fig. 4. Aortic (upper) and P.C.P. (lower) showing effect of aortic compression. Discussion in text (expt. DD-5).

the change could not be assigned to transmission of pressure from collateral branches. Opposed to the idea that a significant collateral supply exists are *a*, the recent observation of Tennant and Wiggers (1935) that recognizable contractions in the ischemic area discontinue approximately within a minute after occluding a main branch, and *b*, our own observations that the flow of blood from the peripheral end of a major coronary vessel is extremely small. In the experience of workers in this laboratory, only an occasional drop flows from a cannula in the peripheral end of the *anterior ramus descendens* of the dog, the total flow per minute being less than 1 cc. Flow rates which even approach those reported by Anrep and Häusler (1928) have never been encountered. These must have been recorded from a hound with unusually developed collateral circuits.

Our analysis of optical tracings (e.g., fig. 2A) precludes attributing the rise of peripheral coronary pressure prior to ejection (A-C) to trans-

mission of pressure from collaterals. The possibility does exist however that the rising systolic plateau (*D-E*) may be due to such a transfer of collateral pressure. The fact that this slope increases both when the areas contract and when aortic pressures mount (fig. 4) might be cited to support such a possibility.

More direct evidence on these points was obtained by studying changes in P.C.P. in the *anterior ramus descendens* following additional temporary ligation of the left circumflex and/or right coronary branches. Such studies were complicated by immediate changes in contraction of the ventricles and by the rapid development of ventricular fibrillation. Despite such difficulties and numerous expected failures, successful results were obtained in 5 dogs. The records of figure 2 illustrate the results of one such experiment. Record A represents a control which has already been analyzed. Without disturbing the manometers, the left circumflex branch was abruptly occluded by application of a special clamp. Record B was taken as quickly as possible thereafter. The aortic pressure curve displays typical effects of occlusion described by Orias (1932, 1934), among them the relatively small fall in aortic blood pressures and the marked reduction in duration of systolic ejection while the heart cycle remains constant. The coronary diastolic pressure decreased 3 mm. and systolic pressure, 6 mm. After occlusion of less than a minute the clamp on the left *circumflex ramus* was released and within another minute curve C was taken. It is practically identical with that of curve A as regards contour, amplitude and time relations. Then, the right coronary was similarly occluded near its origin and curve D taken. It shows no significant changes of any sort.

These and many similar tests showed clearly that compression of the right coronary artery is without effect upon peripheral pressure in the *ramus descendens anterior*; occlusion of the left circumflex branch on the other hand reduces coronary systolic pressure and modifies the form of the curve. The bulk of evidence distinctly favors the view that the changes noted result chiefly and perhaps entirely from altered contraction of the left ventricle, for 1, the lowered coronary systolic pressure (record B) is chiefly due to a smaller initial rise which transpires before any transmission of collateral pressure could have taken place, and 2, the systolic plateau shows an actual increase in the rate of rise, not a decrease, as would be expected if a collateral transfer of pressure had been abrogated.

The slightly rising plateau therefore remains difficult to interpret. The possibility must still be considered that it represents a slight transfer of pressure directly from the ventricular cavities. Such an interpretation would give a function to the communicating channels described by Wearn et al. (1933) and would account for the appearance in capillaries of particulate matter injected into the ventricles when the coronaries are perfused.

from an extraneous source (Bohning, Jochim and Katz, 1933), without necessarily demonstrating the efficiency of such a circulation.

On the basis of such studies the conclusions are reached that the transfer of pressure from collateral vessels plays no significant part in determining the contour or magnitude of peripheral coronary pressure pulses and that the systolic increase in peripheral coronary pressure is chiefly due to muscle tension rather than to changes in muscle length.

Peripheral coronary pressure variations and the appraisal of peripheral coronary resistance. Numerical values for the P.C.P. changes expressed in millimeters mercury and referred to zero at the aortic cannula tip are inscribed directly on curves presented in our illustrations. They indicate the order of magnitude generally found, although considerable difference occurs, particularly in the systolic maximum pressure. Such values doubtless represent the actual maximal pressures developed peripherally to an occlusion under given dynamic conditions. If, however, they also indicate the extreme magnitude of the systolic increase in resistance under normal conditions, then the systolic pressure-difference in the large coronary vessels is great enough to cause a much larger systolic flow than observations by flow-recorders have indicated (Wiggers and Cotton, 1933). A suspicion that such inferences are questionable was aroused by the observations of Anrep and Saalfeld (1933) that when auto-perfused coronary vessels are briefly clamped during systole, the peripheral pressure holds at far higher levels than indicated by our figures. While we have found by repetition of their experiments that the holding level is always distinctly below aortic systolic pressure, it greatly exceeds the pressure maxima indicated in direct P.C.P. curves. Confirmatory evidence is given in the observations that after sudden compression of a coronary branch, the peripheral systolic pressure developed in subsequent beats depends upon the diastolic level from which they start. Thus in the record of figure 3 B, the systolic height decreases progressively in beats 1, 2 and 3. Observations in other experiments showed that even a greater systolic elevation of the curve occurred in still earlier beats, as, for example, the one sketched upon the record. If such beats are enlarged by projection and then redrawn to identical coördinates, their form is found to be unchanged. Such results clearly show that the degree to which the coronary vessels and their branches are filled affects the magnitude of the pressure increment during systole under identical dynamic conditions of cardiac action, but does not alter their form.

The conclusion logically follows that optically recorded curves of P.C.P. picture the *sequential changes in peripheral coronary resistance* correctly as regards time relations and relative magnitude, but they cannot be used quantitatively to appraise the maximum systolic resistance under natural conditions.

We are able to interpret this entirely unforeseen situation in only one way, viz., by postulating a disproportion between the systolic back thrust of blood from the minute vessels and the elastic accommodative capacity of the coronary system involved.

Circumstantial experimental evidence supports this view. The volume-elasticity relations at internal coronary pressures from 20 to 180 mm. Hg were studied immediately after our experiments, in 14 hearts. The method first used consisted in blocking the capillary bed supplied by the *ramus descendens anterior* by perfusion with a dilute suspension of lyco-podium. The vessel was connected to a horizontal micropipette and

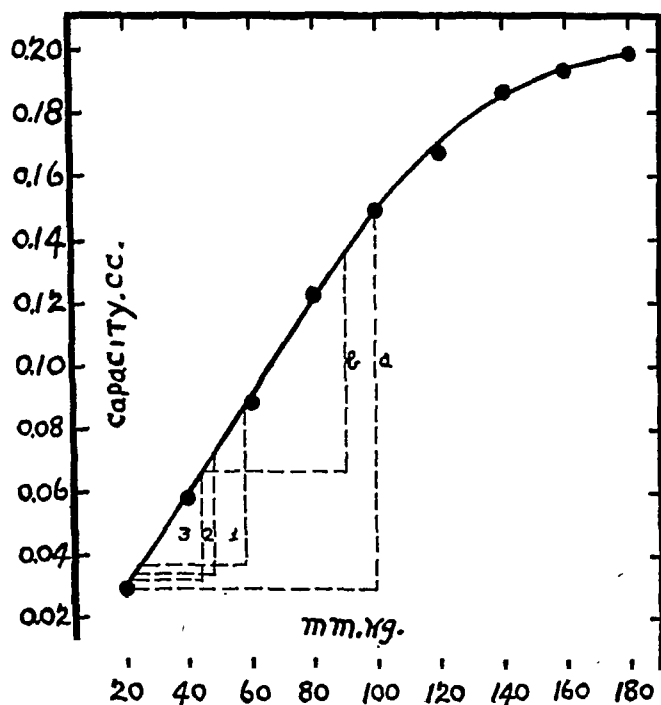


Fig. 5. Plot showing volume-pressure relationships in ramus descendens anterior. Discussion in text.

manometer, and volume-pressure relations were determined. After such treatment blockage was rarely complete for aqueous fluids, but when the vessels and apparatus were filled with mercury, pressures generally held.

Volume-pressure relations of the *anterior descending ramus* and its branches in hearts weighing about 100 to 150 grams are shown by a composite curve in figure 5. Examination reveals that a volume increase of about 0.12 cc. is required to produce a pressure rise from 20 to 100 mm. (line *a*). Similar tests with the optical manometers used showed that introduction of 0.0035 cc. sufficed to cause a similar rise in pressure. Applying such figures it becomes apparent that a pressure of 100 mm.

could only develop in the occluded coronary vessels provided the volume increased at least 0.12 cc. and that registration of the full change by our manometer would require only a trifle more, i.e., a total volume of 0.1235 cc. The only source for this added blood in occluded coronary territory would be by squeezing blood back from minute vessels during systole; a supply from collaterals can at once be eliminated for the major elevation of pressure precedes the rise of aortic pressure. By drawing on the curve of figure 5 vertical lines, 1, 2, 3, the lengths of which correspond to the pressure increases shown in similarly designated beats of figure 3, it becomes apparent that the capacity change and presumably the systolic backflow is of the order of only 0.03 cc.; further that only slight differences in diastolic coronary pressures (i.e., distention) are required to increase the magnitude of the backflow denoted successively by lines 3, 2 and 1. It thus becomes quite possible that in this experiment sufficient backflow to elevate pressures to 90 mm. could have occurred only when the coronary system was distended by considerably higher diastolic pressures (*circa* 45 mm.). This hypothetical condition is illustrated by the vertical line *b*.

Observations such as these suggest that, as far as the *anterior ramus descendens* territory of the dog is concerned, the systolic backflow is much less than generally believed. When a peripheral coronary vessel is perfused with Locke's solution at approximately their diastolic pressures (*circa* 20 mm.) jets of red blood can be seen to enter the cannula during each systole and to leave during each diastole. This phenomenon which attracts the attention of all workers leaves an exaggerated impression of backflow for it must be remembered that blood diffuses rapidly into adjacent saline and rarely shoots much beyond the tip of a cannula, the total capacity of which is only 0.1 cc. Experimental estimates by means of a flow recorder still to be described indicate an actual to and fro movement of about 0.04 cc.

SUMMARY AND CONCLUSIONS

In order to study the phasic changes in peripheral coronary resistance qualitatively and quantitatively, pressure changes in a peripheral coronary branch were recorded optically by two procedures.

Such records indicate that our current conceptions regarding the time relations, character and magnitude of peripheral coronary resistance require some revision:

1. Normally, the peripheral coronary pressure (P.C.P.) increases quickly during isometric contraction and the first moments of ejection, rises more gradually to a summit during the shortening phase, decreases abruptly during isometric relaxation and is influenced but little by subsequent lengthening of ventricular muscle.

2. Such time relations together with the demonstrations *a*, that P.C.P.

curves are not materially affected when the regions involved extend instead of shorten (ischemia), and *b*, that at constant diastolic pressures, systolic coronary pressure increases proportionately to systolic aortic pressure, when the latter rises, indicate that intramural and intraventricular tension rather than muscle fiber length predominantly determines the resistance.

3. The fact that the systolic maximum pressure is reduced somewhat when the involved muscle-area extends instead of shortens can be interpreted to mean either that muscle shortening is normally of supplementary assistance or that stretching increases the capacity of the coronary branches sufficiently to prevent full development of systolic pressure.

4. Peripheral coronary resistance is not affected to any discoverable extent by transmission of pressure from collateral branches because *a*, the magnitude of flow from an open peripheral ramus is very small; *b*, the steep and major rise of P.C.P. occurs prior to development of maximum aortic pressure, and *c*, clamping of the right or/and left circumflex rami produce no phasic changes in resistance and only such deviations in magnitude as can be better explained by concurrent changes in the dynamics of ventricular contraction.

5. In beats equivalent as regards contractile force, the systolic pressure maximum reached depends upon the diastolic pressure level from which a beat starts, i.e., upon the degree of coronary filling. Volume-elasticity studies of the coronary system, interpreted in conjunction with pressure changes and flow determinations, strongly suggest that the systolic back-flow is of the order of 0.03 cc. which is considerably less than usually stated. Since this backflow is less than that required for development of the total pressure of which the myocardium is capable, pressure curves recorded from a peripheral ramus do not allow an appraisal of the maximum resistance developed under natural conditions of coronary distention.

The facts presented are of fundamental importance in understanding phasic changes of coronary flow and in interpreting the actions of drugs upon the intact coronary circulation.

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THE SO-CALLED NORMAL ALCOHOL OF THE BODY¹

R. N. HARGER AND ANNA L. GOSS

From the Department of Biochemistry and Pharmacology, Indiana University School of Medicine, Indianapolis, Indiana

Received for publication March 4, 1935

The statement has frequently been made that ethyl alcohol is a normal constituent of animal tissues and fluids. This idea was first advanced by Ford (1) in 1858. In the intervening seventy-seven years over a score of investigations upon this question have been recorded, practically all of which support Ford's conclusion regarding normal alcohol. In these investigations body fluids or tissues were distilled and these distillates, after more or less concentration and purification, were analyzed for "alcohol" by one of several methods. Reduction of dichromate was employed as the analytical procedure in thirteen of the studies (references 2 to 14); formation of acid after oxidation was used in three investigations (15), (16), (17); two groups (19), (20) employed the interferometer; and modifications of the Zeisel alkoxy method were used in two investigations (20), (21). None of these methods is specific for ethanol.

In the investigations cited most of the figures reported as alcohol range between one and ten milligrams per hundred grams of tissue or body fluid. Although these quantities are too small to have any connection with the problem of inebriety they are of the same order of magnitude as the normal figures for a number of body substances which are determined every day in clinical laboratories, and therefore, if correct, may be of biological significance and perhaps of clinical interest.

Only two papers report no "normal alcohol."² They are the studies of Lenoble and Daniel (7) and Kridelka and Bohet (11), using spinal fluid and blood respectively. However, these workers simply tried the Nicloux

¹ Part of this work was presented before a joint meeting of the sections on Biological Chemistry and Medicinal Chemistry of the American Chemical Society on April 1, 1931 at Indianapolis, Ind. and a preliminary report appeared in *J. Indiana State Med. Assn.* 25: 384, 1932.

² Gettler, Niederl, and Benedetti-Pilcher (21) quote Umber (*Ztschr. Klin. Med.* 39: 12, 1900), and Arnheim and Rosenbaum (*Ztschr. Physiol. Chem.* 40: 220, 1904), as denying that alcohol is normally present in the body, but a perusal of the papers quoted fails to confirm this statement. These workers simply investigated the question whether or not blood or body tissues are capable of fermenting or consuming glucose in vitro, and they made no effort to study the question of "normal alcohol."

dichromate method using small quantities of material (2.5 to 5.0 cc.), which procedure would hardly allow the detection of concentrations of alcohol lower than about ten milligrams per hundred grams.

A consideration of the existing literature raises two questions:

1. *Is ethyl alcohol present in the concentrated distillates from body tissues and fluids?* Several of the studies describe the isolation of a material which supported combustion, reduced chromic acid with the formation of aldehyde and organic acid, and gave a positive iodoform reaction. J. Bechamp (15) reported the isolation of sodium acetate after oxidizing the alcohol-like substance, while Maignon (3) stated that he converted the acetic acid formed into ethyl acetate, ethyl butyrate, and cacodyl. Taylor (22) applied the Buchner test and recorded the formation of the ethyl ester of p-nitro benzoic acid. Using an ingenious micro distillation apparatus and other micro methods Gettler, Niederl, and Benedetti-Pilcher (21) reported the isolation from body tissues of an anhydrous fluid which boiled at around 78°C., having the carbon content of ethyl alcohol, and which was converted into ethyl iodide and ethyl benzoate. This evidence strongly points to the presence of ethyl alcohol in some of the concentrates obtained.

2. *Assuming that ethyl alcohol was found in some of these distillates was it present at the time of death?* The evidence on this point is by no means conclusive. The traces of alcohol reported might result from postmortem fermentation since the conversion of a small fraction of the tissue glucose to alcohol would account for the results obtained. Furthermore, the body contains several substances having ethoxy groups which might be hydrolyzed during the distillation or subsequent concentration.

We have attempted to approach this problem from a somewhat different angle than has been employed by previous investigators. The procedure adopted was suggested as a result of our observation that if the steam distillation of body tissues or fluids was prolonged beyond the point where all traces of pre-formed alcohol would be removed, *a volatile reducing substance continued to come over in the distillate in quantities almost as great as in the beginning.* This indicated that a part, at least, of the reducing substance obtained was either a material of low volatility, or the result of decomposition during the distillation. Accordingly, we steam-distilled specimens of tissues and body fluid collecting the distillate in two successive portions, each having a weight equal to that of the fluid or tissue used and after purifying and concentrating these distillates we determined their reducing power by a modified dichromate method. By adding alcohol (1 mgm. per 100 g.) in control experiments we showed that the added alcohol appeared almost quantitatively in the first fraction of the distillate. *Therefore, any reducing material appearing in the second fraction of the distillate could not be alcohol existing in the tissues before distillation. Since the first frac-*

tion must have contained at least as much of this material, which could not have been normal alcohol, as the second, the normal alcohol could not have been more than the difference between the reducing substance in fractions one and two. While the method employed is not specific for ethanol but simply determines volatile reducing substances secured by the procedure employed, it at least gives *maximum* figures for the amount of ethanol present. Although our studies have not settled the question regarding what fraction, if any, of the volatile reducing substances obtained is made up of ethanol, we believe that the results do contribute to a solution of the problem of "normal" alcohol in that they show that most of the figures reported by previous investigators are far too high.

Before analysis the distillates were purified and concentrated by fractionating first from acid and then from alkaline silver nitrate. To minimize the evolution of reducing substances less volatile than alcohol we distilled off only one-fifth of the volume in each fractionation. Control tests with small amounts of alcohol showed a recovery of about 87 per cent by this process. Besides resulting in lower figures for "alcohol" this procedure reduced the number of distillations to secure the desired concentration.

With urines and a few tissues we added a further purification step in which the final 10 cc. obtained by the process described above were refluxed with alkaline mercuric chloride, the procedure being a modification of the method of Gorr and Wagner (23) for the removal of acetone from ethanol.

Preliminary experiments confirmed the findings of Pringsheim (4) that there is a post-mortem increase in the volatile reducing substance in tissues. Consequently, we made an effort to steam-distill the material as soon as possible after the death of the animal, or removal of blood or urine from living subjects. Since distilled water on standing develops a small amount of volatile reducing substance, only freshly distilled water was used, and in generating steam the first portion of the steam was discarded.

PROCEDURE. One hundred grams of urine, blood, or hashed tissue were placed in a flask and to this were added 100 cc. of freshly distilled water, 0.5 gram of tartaric acid, and a small piece of paraffin. The flask was heated in boiling water and its contents steam distilled in the usual manner, the vapor evolved passing to the condenser through a column containing a Kjeldahl connecting bulb. The distillate was collected in two successive 100 cc. portions. In a few cases a third or fourth 100 cc. fraction was collected. Each 100 cc. portion was then transferred to an ordinary 250 cc. distilling flask and acidified with 0.2 cc. of concentrated sulfuric acid. The flask was connected to a small vertical condenser, and its contents boiled over a free flame until exactly 20 cc. of distillate had been collected. This distillate was then transferred to a 100 cc. distilling flask and two 10 cc. portions of distilled water used to rinse out the condenser

and receiving flask, these washings being added to the 20 cc. of distillate. Three cubic centimeters of 5 per cent silver nitrate were next added, followed by 2.5 cc. of 10 per cent sodium hydroxide. This flask was connected with the same type of condenser used in the first distillation and the contents (45.5 cc.) boiled over a free flame until exactly 9 cc. of distillate were collected. Any alcohol adhering to the condenser tube was rinsed out with two 0.5 cc. portions of distilled water. The resulting 10 cc. thus represented a ten-fold concentration of the original steam distillate. After mixing, 5 cc. were withdrawn and analyzed for reducing power by a modified dichromate method described elsewhere (24). In the case of human blood, 50 cc. samples were employed, the volume of distillates and reagents being reduced to one half, and the final distillate of 5 cc. was all used for the determination of reducing material. With urine and a few tissues the final 10 cc. obtained as outlined above were transferred to a flask to which were then added 5 cc. of 5 per cent mercuric chloride, 3 cc. of 3 per cent sodium hydroxide and two 1 cc. portions of water, the last being used to rinse out the receiving tube. The flask was connected to a reflux condenser by means of a ground glass joint and the contents boiled for 15 minutes. At the end of this time the flask was cooled and the condenser tube rinsed out with 2 or 3 cc. of water. The flask was then connected to a condenser for distillation and boiled until exactly 10 cc. of distillate had collected; 5 cc. of this distillate were then analyzed for reducing power and the remainder of the distillate tested for acetone by the method of Behr \acute{e} and Benedict (25). In all cases no acetone was found although it was always present in the concentrates from urines not treated with alkaline mercuric chloride. Control experiments showed that this refluxing with alkaline mercuric chloride served to remove acetone without destroying ethanol, whereas refluxing with alkaline silver nitrate failed to remove acetone. Before each distillation the condenser tubes and distilling flasks were cleaned by being boiled in concentrated nitric acid after which they were carefully rinsed with distilled water. This was done because a volatile lipoid-like material frequently was deposited in the condenser tubes during concentration of the tissue distillates.

RESULTS. 1. *Reducing material in successive portions of distillate.* Hundred gram samples of human brain or beef liver were steam-distilled as described above, several portions of distillate being collected. Five cubic centimeter specimens of these distillates were analyzed directly for reducing material, and the remaining 95 cc. portions were concentrated ten-fold as described, and then analyzed. The results are given in table 1. It will be noted that the presence of reducing material in the distillates persisted long after all pre-formed alcohol should have been removed, and that the concentration process removed a great deal of the reducing material.

2. *Recovery of added alcohol.* We first determined the amount of alcohol recovered in the concentration procedure. One milligram of alcohol was added to 100 cc. of freshly distilled water and this was concentrated in the two steps, as described, first from acid and then from alkaline silver nitrate. In six experiments analysis of the final concentrates of 10 cc. showed a recovery of 0.894, 0.874, 0.890, 0.874, 0.824, and 0.844 mgm., respectively, or an average of 0.867 mgm. Thus an average loss of 13.3 per cent occurred during concentration.

We next tried adding 1 mgm. of alcohol to 100 grams of hashed beef liver or animal blood³ and determined the amount of alcohol recovered when these tissues were steam distilled, concentrated and purified in the manner described. Controls on the same tissues were run simultaneously.

TABLE 1
Reducing substance from successive fractions of distillate

TISSUE	FRACTION OF DISTILLATE	DICHROMATE CONSUMED BY DISTILLATE FROM 100 GRAMS OF TISSUE*	
		Analyzed directly	Purified and concentrated
		cc. of 0.0434 N $K_2Cr_2O_7$	cc. of 0.0434 N $K_2Cr_2O_7$
Human brain.....	First 100 cc.	1.71	0.72
Human brain.....	Second 100 cc.	0.69	0.334
Human brain.....	Third 100 cc.	0.55	0.226
Beef liver.....	First 100 cc.	2.72	0.948
Beef liver.....	Second 100 cc.	2.14	0.440
Beef liver.....	Third 100 cc. (rapid distillation)	2.72	
Beef liver.....	Fourth 100 cc. (very rapid distillation)	7.50	

* One cubic centimeter of 0.0434 N dichromate is consumed by 0.5 mgm. ethyl alcohol.

In four experiments the figures for the recovery of alcohol in the first 100 cc. of distillate were respectively⁴ 0.998, 0.923, 0.836 and 0.883 mgm. or an average of 91.0 per cent. The figures for alcohol recovered in the second 100 cc. of distillate were respectively⁴ 0.01, 0.20, 0.02 and 0.00 mgm. of alcohol or an average of 5.8 per cent. When alcohol was added and the material allowed to stand for two hours before being distilled, the yield of alcohol was⁴ 71.2 and 61.5 per cent in the first 100 cc. and 3.6 and 16.4 per cent in the second 100 cc. Ford (1) and Fleischman and Trevani (26) noted that blood is able to decompose alcohol in vitro. The addition of but one milligram of alcohol per hundred grams of blood or tissue caused

³ The blood was preserved with 1 per cent of KF, 2 H₂O.

⁴ Corrected for a loss of 13.3 per cent during concentrations.

a great rise in the reducing material appearing in the first 100 cc. of the distillates, the increase ranging from almost threefold to elevenfold.

3. *Effect of delay before analysis.* Hog livers were removed from the animals and immediately brought to the laboratory. The tissue was hashed and one hundred gram portion was steam distilled as quickly as possible. The remainder was placed in a closed vessel in a well cooled refrigerator and after intervals of twenty-four hours and six days hundred gram portions were steam-distilled. Prior to analysis the steam-distillates were purified and concentrated ten-fold as usual. The consumption of 0.0434 N. dichromate by the first 100 cc. portion of distillate was as follows: liver A, one-half hour, 1.075; twenty-four hours, 1.688; six days, 20.80; liver B two hours, 0.828; twenty-four hours, 1.910; six days, 53.32. Delay before distillation caused very little change in the reducing material appearing in the second 100 cc. of distillate.

4. *Reducing material obtained upon prompt distillation of fresh body tissues and fluids.* In these experiments special precautions were taken to steam distil the material as quickly as possible after the death of the animal or the removal of blood or urine from living subjects. In each case a 100 gram sample was steam distilled except with the human bloods where a 50 gram sample was used. The two fractions of distillate obtained from each sample were then concentrated and purified as described above under *procedure*. In the case of human urines and also with certain tissues the distillate from the alkaline silver nitrate treatment was further purified by refluxing with alkaline mercuric chloride as described above. A large number of tissues, bloods and urines were put through this procedure. Table 2 records typical results from these experiments. In order to save space we have included only about half of the results since they were fairly uniform. The results given include the maximum variations found.

DISCUSSION. While it is true that the method of analysis employed in this study is not specific for ethanol, the same criticism applies to all other methods for estimating ethanol, of which the authors are aware. Theoretically it might seem that greater accuracy would be attained by estimating ethanol as a conversion product such as the iodide or acetic acid. However, the much higher figures reported elsewhere when these methods were applied to this problem would indicate that reactive substances other than ethanol were present.

It might be argued that the continued evolution of reducing material when the steam-distillation is prolonged may mean that traces of normal alcohol are tenaciously held by the tissues and therefore not all removed in the first fraction. This theory would appear to be disproved by our experiments on the recovery of added alcohol. Here, after adding only one milligram of alcohol to 100 grams of body tissue, we found that practically all of the added alcohol appeared in the first fraction of the distillate.

Even when the alcohol was in contact with the tissue (blood) for two hours there was very little increase in the amount of reducing material in the second fraction of distillate.

TABLE 2

Reducing material in successive distillates from body tissues and fluids

	DICHROMATE CONSUMED BY DISTILLATE FROM 100 GRAMS OF TISSUE OR FLUID*			MAXIMUM FIGURE FOR NORMAL ALCOHOL (a-b) $\times 0.5 \times$ $1/0.867\dagger$
	First 100 cc. of distillate (a)	Second 100 cc. of distillate (b)	Difference (a-b)	
	cc. of 0.0434 N $K_2Cr_2O_7$	cc. of 0.0434 N $K_2Cr_2O_7$	cc. of 0.0434 N $K_2Cr_2O_7$	mgm. per 100 grams
Dog A:				
Blood.....	0.236	0.220	0.016	0.009
Liver.....	0.422	0.218	0.204	0.199
Kidney.....	0.366	0.290	0.076	0.044
Brain.....	0.506	0.422	0.084	0.049
Muscle.....	0.392	0.328	0.064	0.037
Dog B:				
Blood.....	0.200	0.267	None	None
Brain.....	0.734	0.530	0.204	0.119
Liver.....	0.760	0.370	0.390	0.227
Kidney.....	0.377	0.134	0.243	0.142
Muscle.....	0.540	0.150	0.390	0.227
Human blood, subject:				
H. W. B.....	0.167	0.160	0.007	0.004
R. N. H.....	0.180	0.173	0.007	0.004
D. J. W.....	0.217	0.293	None	None
H. R. H.....	0.347	0.300	0.047	0.027
Beef liver†.....	0.272	0.103	0.169	0.085
Beef kidney†.....	0.179	0.075	0.104	0.060
Human urines:‡				
Specimen 1.....	0.385	0.281	0.104	0.060
Specimen 2.....	0.308	0.127	0.181	0.104
Specimen 3.....	0.380	0.262	0.118	0.068
Specimen 4.....	0.498	0.177	0.321	0.185
Specimen 5.....	0.342	0.134	0.208	0.120
Specimen 6.....	0.241	0.098	0.143	0.083

* One cubic centimeter of 0.0434 N dichromate is consumed by 0.5 mgm. ethyl alcohol.

† Corrected for loss of 13.3 per cent of alcohol during concentration.

‡ Refluxed with alkaline mercuric chloride.

Our assumption that the maximum figure for pre-formed alcohol is represented by the excess of reducing material in the first fraction of distillate over that in the second fraction probably errs in the positive direction,

because the third fraction contained somewhat less reducing material than the second, indicating that still more of the reducing material of the first fraction was not due to pre-formed alcohol.

Finally, our conclusion that the normal alcohol, if any, is much smaller than the figures usually given is supported by two large scale experiments reported in the literature. Ford (1) distilled fifty pounds of ox lung and obtained no alcohol. He attributed this negative result to the fact that the experiment was done during hot weather. In the large scale experiment recorded by Gettler, Niederl, and Benedetti-Pilcher (21), 28 kilos of pigs' brains were employed and the figure obtained for "alcohol" was only 0.07 mgm. per 100 grams. This very low figure is in marked contrast to the other figures reported by these investigators using smaller quantities of tissues. Thus their maximum figure for human liver "alcohol" is *eighty times greater* than this figure for pigs' brain!

SUMMARY AND CONCLUSIONS

1. Fresh specimens of urine, blood, and body tissues were promptly steam-distilled, two or more successive fractions of distillate being collected, each fraction having a weight equal to that of the specimen used. Each fraction of distillate was then purified and concentrated ten-fold, and these concentrates tested for reducing power by a micro dichromate method.

2. In all cases the first fraction of distillate contained a small amount of reducing material, which, expressed as ethanol, ranged from 0.09 to 0.38 mgm. per 100 grams of body tissue or fluid.

3. These small quantities of reducing material in the first fraction of distillate were shown to be largely not pre-formed ethanol because succeeding fractions of the distillate contained almost as much reducing material.

4. In control experiments it was shown that a trace of added ethanol (1 mgm. added to 100 g. of body tissue or fluid) was almost all recovered in the first fraction of the distillate.

5. Since the reducing material in the second fraction of the distillate could not be pre-formed alcohol, and since the first fraction of distillate must have contained at least as much of this reducing material, which also was not normal alcohol, then the maximum figure for normal alcohol may be represented as A-B where A and B represent the quantities of reducing material in the first and second distillate fractions respectively.

6. Our results with fresh tissue, calculated on the basis just described, give maximum figures for normal alcohol, expressed as milligrams of ethanol per 100 grams of body tissue or fluid, as follows: blood 0.0 to 0.027, brain 0.049 to 0.119, liver 0.085 to 0.227, kidney 0.044 to 0.142, muscle 0.037 to 0.227, and urine 0.060 to 0.185.

7. When body tissues were allowed to remain in a refrigerator for some time before being steam-distilled, an increase was noted in the quantity of

reducing substances appearing in the first fraction of distillate. Thus, after twenty-four hours the quantity of reducing substance was almost doubled, and after a delay of six days the increase was more than twenty-fold.

8. The normal concentration of body ethanol, if any, is very much smaller than has been reported by most previous investigators.

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RATES OF RESORPTION IN THE GALL BLADDER

FURTHER EXPERIMENTS WITH METHYLENE BLUE ON RABBITS

BÉLA HALPERT, PAUL A. O'CONNOR AND WILLIAM R. THOMPSON

From the Department of Pathology, Yale University School of Medicine

Received for publication April 19, 1935

In former experiments with methylene blue on rabbits it was shown that it is possible to calculate the mean rate of flow of bile through the cystic duct and the amount of fluid resorbed from the gall bladder per unit of time (1). It was observed that when the bile was secreted against a constant hydrostatic pressure of 50 mm. greater than that of the atmosphere, the gall bladder resorbed approximately half the volume of its contents per hour. The present studies were designed to determine whether the rates of resorption from the gall bladder are influenced by pressure. Thus experiments were planned in which bile was secreted against a constant hydrostatic pressure of 0, 75 and 100 mm., respectively, greater than that of the atmosphere.

METHOD. The method used was identical with that applied by Halpert, Thompson and Marting in their experiments (1). With the animal under ether anesthesia, a glass cannula was tied into the common bile duct close to its termination in the duodenum. The cannula was joined with a rubber tube to the inlet of a glass trap connected to a remote gas chamber with a hydrostatic pressure maintained at the desired level. After the proper adjustments were made, the bile was carefully drained from the trap and discarded. Collections were then commenced over successive intervals of one-half hour. Immediately after the first of these was started 2 ml. of a 1 per cent solution of methylene blue per kilogram of body weight of the animal were injected into the marginal vein of the left ear. The volume of bile in each half hour collection was measured and its methylene blue content determined. The experiments were concluded at the end of three hours, at which time the entire content of the gall bladder was removed and its volume and methylene blue concentration determined (2).

As in former experiments (1) the calculations were based on the assumption that in any given interval during the experiment the volume of bile which entered the cystic duct was approximately proportional to that which left the common bile duct, and that the amount of bile withdrawn from the common bile duct was replaced by an equal volume of bile of the

same methylene blue concentration as that entering the cystic duct. However, essentially the same results are obtained from an alternative assumption that the rate of flow through the cystic duct is independent of the rate of flow through the common bile duct.

EXPERIMENTAL DATA. The pertinent data of the experiments are summarized in table 1. It may be seen that there are considerable individual

TABLE 1

The transportation of methylene blue in the bile of the rabbit following intravenous administration of the dye (2 ml. of a 1 per cent solution per kilogram of body weight)

RABBIT (MALES)		HYDRO- STATIC PRESSURE	VOLUME OF BILE OBTAINED FROM		TOTAL METHYLENE BLUE IN BILE FROM		MEAN RATE OF FLOW OF BILE THROUGH THE CYSTIC DUCT (r)	$\frac{r}{v_0}$
Num- ber	Weight		D. chole- dochus (V)	Gall bladder (v_0)	D. chole- dochus (M)	Gall bladder (m)		
	kgm.	mm.	ml.	ml.	mgm.	mgm.	ml./hr.	hr. ⁻¹
2	2.5	0	19.6	1.0	5.42	0.633	0.746	0.762
4	3.1	0	50.4	1.7	12.67	0.155	0.213	0.121
5	2.9	0	31.9	1.3	11.33	1.22	1.130	0.818
6	3.2	0	24.4	2.8	9.01	1.10	1.004	0.355
Mean.....			31.6	1.7	9.61	0.78	0.77	0.51
a.d.....			9.6	0.6	2.39	0.38	0.29	0.28
7	2.6	75	33.8	3.9	8.37	2.50	2.15	0.516
8	2.70	75	29.0	2.1	11.20	0.656	0.581	0.270
9	2.97	75	29.7	1.5	7.91	0.694	0.904	0.580
11	2.97	75	48.4	2.0	2.53	0.770	0.511	0.244
Mean.....			35.2	2.4	7.50	1.16	1.04	0.40
a.d.....			6.6	0.8	2.49	0.48	0.56	0.15
12	2.85	100	27.7	2.7	7.004	1.125	1.44	0.548
14	2.80	100	29.7	3.7	5.383	0.748	1.38	0.372
15	3.1	100	38.0	3.1	8.237	0.896	1.39	0.444
17	3.0	100	14.3	1.9	6.180	1.9	1.34	0.771
Mean.....			27.4	2.8	6.70	1.17	1.39	0.53
a.d.....			6.6	0.6	0.92	0.80	0.03	0.13

variations in the volume (V) and the methylene blue content (M) of the bile obtained from the ductus choledochus during the experimental period. Similar individual variations were noted in the volume (v_0) and the methylene blue content (m) of the bile obtained from the gall bladder. The volume of bile and its methylene blue concentration in the gall bladder as well as the mean rate of flow of bile through the cystic duct (r) appeared to be somewhat greater in the experiments with higher pressure. How-

ever, the relative rates of resorption from the gall bladder $\left(\frac{r}{V_0}\right)$ were about the same in the experiments at 0, 75, and 100 mm. of hydrostatic pressure, i.e., approximately one-half the volume of the contents of the viscus per hour.

SUMMARY

Methylene blue was given intravenously to rabbits and the bile, secreted against a constant hydrostatic pressure respectively 0, 75 and 100 mm. above the atmosphere, was collected from the common bile duct and the contents of the gall bladder were removed at the end of the experiment. The methylene blue content of each sample of bile was then determined and the amount of bile which entered the gall bladder during the experimental period was estimated. The data thus far obtained indicate that the rate of resorption of fluid from the gall bladder was greater in the experiments with higher pressure. However, the ratios of these rates to the volumes of gall bladder contents were about the same, approximately half the volume of the contents of the viscus per hour.

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THE WATER AND CHLORIDE EXCRETION OF DECEREBRATE CATS

MARGARET SUMWALT, W. H. ERB AND H. C. BAZETT

From the Department of Physiology, University of Pennsylvania Medical School

Received for publication April 10, 1935

That diabetes insipidus may be experimentally induced by damage to the hypothalamus or hypophysis is now generally recognized. (For a short review, see Leschke, 1933.) So is the conception that the control over water balance normally exerted from this region is humoral, rather than nervous. Yet conflicts of evidence occur, even in connection with these well established points; and other details, scarcely less important for visualizing the mechanisms concerned, remain open questions. For example, irritation rather than deprivation of nervous structures is occasionally advocated as the cause of diabetes insipidus; and sometimes polyuria is held responsible for polydipsia, sometimes the reverse.

The study to be reported in the present paper utilized familiar techniques in a combination which, for this field, is somewhat novel. Neglecting the localization problem, we treated the pituitary body with neighboring structures as a unit, and sought to identify the nature of their joint influence upon chloride and water excretion in cats by comparing the effects of three types of operation. The pituitary and hypothalamus were removed from the cranium entirely; or their nervous connections with the rest of the animal were severed with as little disturbance as possible to their blood supply; or they remained *in situ* with nervous and also possible chemical pathways intact. For more complete regulation of the environment, activity, diet, and urine collection of the cats, all of the lesions were studied against a background of decerebration.

METHOD. Our study is based upon 46 operated animals. Three were acute preparations, decerebrated under ether by the guillotine, without aseptic precautions. Their urine was collected through a bladder cannula each half-hour after the operation. The other 43 cats were maintained in a living condition for from 3 to 16 days, while their daily water and chloride excretion were measured for comparison with their daily intake. The three types of operation already outlined divided them into three groups. The lesions will be described with their results. All shared alike in the general treatment which they received. The operative technique described by Bazett and Penfield (1922) was used; except that, for chloroform, dial

combined with nembutal was substituted in the proportions suggested by Bazett, Alpers, and Erb (1933); and the cranial dead space was not invariably filled with wax.

Most of the chronic cats underwent, immediately after the brain operation, a suprapubic cannulation of the urethra with a glass cannula which penetrated the internal sphincter, so that drainage was continuous. (In a few cases not so cannulated, feces and urine were collected together by a funnel, and analyzed together.)

A rubber tube on the urethral cannula conducted urine by gravity flow into a flask. We made no attempt to prevent evaporation or to allow for it, but relied upon the high humidity of the room to keep this loss insignificant. The accumulated 24-hour specimen was collected each morning and measured. Samples were analyzed by the modified Volhard-Harvey titration for urinary chlorides as described by Peters and Van Slyke (1932). It was almost invariably necessary, with the urine of both normal and decerebrate cats, to digest the sample with potassium permanganate over a flame, before the mixture of urine with nitric acid and silver nitrate would be sufficiently colorless for titration.

Previous to operation no attempt was made to regulate the diet of the cats. Their postoperative regime consisted of a daily 200 cc. of milk, which was found to contain 360 mgm. of NaCl if all the chloride content is assumed to have been in this form. This was administered in two doses, morning and evening. The actual daily intake of an animal was sometimes less than this, when his resistance to feeding made it seem wiser to withhold a portion of a meal, or part was lost by vomiting. More than this they received only in a few instances, when we doubled the water content or the salt content of a feeding or two, with several cats, to see whether a change in the fluid/salt ratio of the diet would alter the type of excretion. Since these sporadic experiments slightly mar the uniformity of regime, the number of cat-days upon which such special rations were given appears in table 1. The number was small relative to the total number of experimental days; and no effect which could be attributed to the special feedings was ever observed to follow them. (For instance, the volume of urine excreted increased as often as it decreased, during a period in which extra water was given.) Hence the results are discussed as though the diet had been uniform. The other features of postoperative care, such as temperature regulation and recording, humidification, and cleaning, followed closely the method described by Bazett, Alpers and Erb (1933).

Our comparison of ingested water and chloride with urinary water and chloride does not pose as a study of water and chloride balance complete. We never recovered from any cat, over a period of several days, as much as he received in that same time (though occasionally, for one or two days, water output exceeded intake). At least half of the ingested fluid was

probably lost by evaporation.¹ When the excreta were collected together, fecal admixture did not appear to increase either volume measurements or chloride measurements significantly. Some chloride may have been lost in the nasal secretions, and by vomiting, in a few cats. At any rate, unmeasured losses must have been roughly the same for all the cats (with one group exception to be mentioned later), and variations among them therefore reflect real differences in their water or chloride balance.

We performed gross but not microscopical autopsies. When the animals died unexpectedly, as they usually did, the incubation which ensued before they were discovered often made the gross autopsies unsatisfactory. The animals are therefore classified according to the intention of the operation, except when autopsy or physiological behavior other than water balance revealed a failure.

There is no definitely known reason why decerebrate cats of any of our three types should not survive for long periods. We made every effort to prolong their lives. The commonest manifest reasons for death were accidents to the heat regulating system, aspiration of vomitus, or infection (cranial or pulmonary). The causes of about 25 per cent of the fatalities were obscure. Despite the diversity of deaths that befell, success in prolonging survival seemed to be curiously correlated with the type of chloride excretion, and this in turn with the kind of section. This suggests that the nature of the lesion may have been responsible for some of the inexplicable deaths and even perhaps indirectly for some of those in which the immediate cause seemed to be apparent. Susceptibility to infection or frequency of vomiting, for instance, may have depended remotely on chloride unbalance.

It is difficult to subject the normal cat to the conditions borne by the decerebrates. Three kinds of approximation to normal controls were studied, and are shown in table 1. One normal cat which was offered the standard diet for four days in a cage, though not in the incubated room, excreted a daily average of 60 cc. of urine with a content of 210 mgm. of NaCl. Another normal cat, kept free in the laboratory, and trained to micturate in a glass crystallizing dish, ate the diet of the experimental animals for two four day periods, with results which closely resembled those with the caged cat. His daily excretion during one of these periods is shown in figure 2 A. During another four day period he was given water *ad lib.*, besides the 200 cc. of milk, in an attempt to compensate him for his greater loss by evaporation as compared with the humidified cats.

¹ Several animals of the "island" type, in which the chloride excretion was unfortunately not being investigated, were sufficiently normal to survive over three weeks, and showed little or no change in weight. In these the volume of fluid excreted averaged half of that ingested. Though the humidity of the room was 80 per cent at 26°, considerable amounts of water could be evaporated at body temperature.

Under these conditions his average daily water and chloride excretion was greater than before: 110 cc. with 325 mgm. As an attempt at a third type of control, a cat was cannulated and kept anesthetized with dial under the same conditions as the decerebrate animals. It survived two and a half days, and yielded figures which do happen to agree fairly well with the caged and free cats'. It could not be considered a normal animal, however.

RESULTS. *Group 1. High section.* Cats in which both nervous and humoral pathways from the pituitary and hypothalamus were to be left

TABLE 1

Group figures for the survival time, chloride excretion, and water excretion of control and decerebrate cats

Their intake was 200 cc. of milk daily, containing 360 mgm. of NaCl, except on a few days when special rations described in the text were given. The number of days upon which the averages for excretion are based are given in adjacent columns. Chloride is given as NaCl.

GROUP	NO. OF ANIMALS	NO. OF DAYS SUR- VIVAL	NO. OF DAYS OF SPECIAL RATION	NO. OF DAYS	MEAN VOL. PER DAY	NO. OF DAYS	MEAN NaCl PER DAY	NO. OF DAYS	MEAN CONC. NaCl
					cc.		mgm.		per cent
Control:									
Caged cat and trained cat....	2			10	62	9	264	9	0.426
Trained cat with water ad lib..	1			3	110	3	325	3	0.295
Dial cat (abnormal).....	1	2		2	43	2	159	2	0.37
Operated:									
Group 1. High section.....	4	14	4	14	45	14	76	14	0.169
Group 2. Low section.....	9	33	9	30	131	29	97	29	0.074
Group 3. Island section.....	30	179	9	155	97	131	129	131	0.133
Long lived.....	13	123	4	102	98	85	154	85	0.157
Short lived.....	17	56	5	53	95	46	82	46	0.086
Small volume.....	8	23	0	20	51	17	68	17	0.133
Large volume.....	9	33	5	33	115	29	91	29	0.050

intact were prepared by a single nearly horizontal section (level 1, fig. 1), which sloped from a point just in front of the posterior colliculi dorsally to the optic chiasm ventrally. Structures above this level were removed. These cats developed at least partial temperature control about two days after operation. They responded to loud sounds, and sometimes violently resisted feeding, cleaning, and weighing. Though tied to their beds, they performed running movements for long periods, punctuated with intervals of complete quiet. Since they suffered the smallest operative damage of any group, they might be expected to resemble normal cats most closely,

and so to constitute an operated control. The result proved otherwise. They differed greatly from the normal cats in two ways: survival less than five days, and a chloride output averaging 76 mgm. as against 264 mgm. in the normal animals. Their water excretion also was lower than normal, though the difference in this respect is less striking.

The results in this group, however, were undoubtedly complicated by the excessive activity of the animals. For instance, dehydration through overventilation must have reduced the available water for excretion. Perhaps the diminutive urine volume, in turn, was responsible for the low chloride excretion; though the normal cats, excreting only 38 per cent more water, eliminated 250 per cent more chloride. Group 1, therefore, failed to furnish preparations which are comparable with the other two groups.

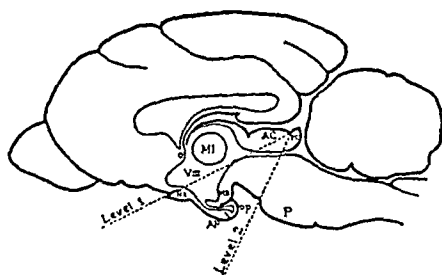


Fig. 1. The two levels of section which were used in decerebrating, shown diagrammatically in relation to the structures which lie in or near the median plane of the cat's brain.

AC—anterior colliculus; PC—posterior colliculus; MI—massa intermedia; V—third ventricle; N2—optic nerve; MB—mammillary body; P—pons; AP—anterior lobe of pituitary; PP—posterior lobe of pituitary.

Group 2. Low section. To ablate the pituitary and hypothalamus, the spatula passed from the same point dorsally as level 1, in a more nearly vertical plane downward to the anterior edge of the pons (level 2, fig. 1), and removed everything anterior to that level. The nine cats prepared in this way resembled group 1 in shortness of life and low rate of chloride excretion. Unlike group 1, they excreted a large volume of water. The average, 131 cc., exceeded the average in any other operated group; and surpassed the control averages, even in that experiment upon a normal cat when water was allowed freely in addition to the standard diet. But average figures do not do full justice to the peaks of water output which they achieved on single days: 260, 250, 230, 220, 200, 200, 190, 160, 40 cc. Four of the nine animals excreted more than 200 cc. a day for two days. Such a rate was never maintained for more than two days. It is not to be expected that on a fixed intake of 200 cc., with simultaneous losses through other channels, a cat could continue to excrete so large a volume. These animals lost weight more rapidly than the other groups. With four animals, the peak excretion occurred during the first or second day, followed

by steady subsidence (fig. 2 C); in another four, it occurred during the third day preceded by considerably smaller volumes (fig. 2 D), and followed by subsidence if they survived through a fourth day. One animal differed from the others in not exceeding 40 cc. for any day of its survival.

We were interested in the rapid onset of polyuria which occurred in four of these cats. The three acute preparations which have been mentioned were therefore studied in this connection. The guillotine method was used in order to obviate all suspicion that any pituitary fragments

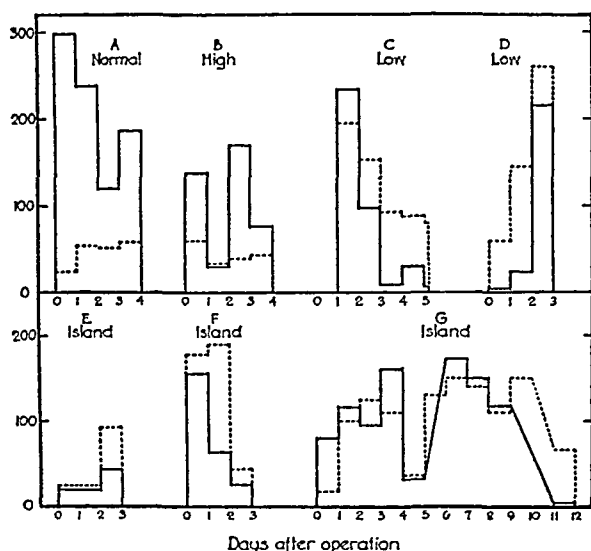


Fig. 2

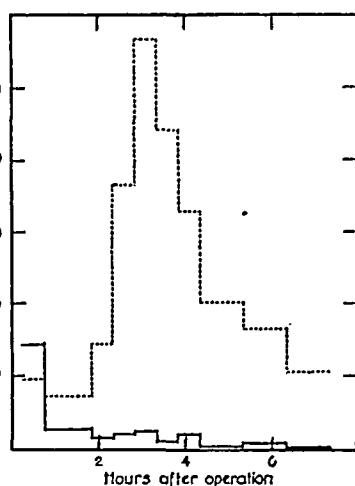


Fig. 3

Fig. 2. Sample individual records, showing types of case histories and the sort of daily variations encountered in a single case. Death occurred on the day following the last collection shown in each case. Solid line = NaCl excretion in milligrams per day. Dotted line = H₂O excretion in cubic centimeters per day.

Fig. 3. Sample record from an acute decerebrate. Urine was collected every half hour, but the units are for daily rates calculated on the basis of these collections. Solid line = milligrams of NaCl. Dotted line = cubic centimeters of H₂O.

remained. Ether was discontinued as soon as decerebration was performed. Within two hours thereafter in all three cats, the chloride rate had fallen considerably. At the end of six hours it was vanishingly small. Meanwhile the volume rate of urine excretion rapidly increased. (See fig. 3 for a sample record, in which the units chosen to express rate are the same as for the chronic cats.) The acute animals were killed at the end of eight or nine hours. Two of the three, by this time, were showing a falling rate of water excretion, and one of them an increasing chloride rate. They had received no food or fluid, either during the experiment, or in the twelve hours preceding it.

The operation undergone by the animals of group 2 was expected to

determine whether ablation of the pituitary and neighboring regions was capable of giving rise to an experimental diabetes insipidus, when there could be no question of irritation to surviving tissues. The three acute cats and eight out of the nine chronic cats did develop polyuria. It was easy to ascertain, either at the time of operation, or at autopsy, that the hypothalamus and all other parts of the brain overlying the sella turcica, down to the pons, had been completely removed. Therefore irritation of these could not have caused the polyuria. Hemorrhage or other damage at the level of section might conceivably irritate tracts or centers in the remaining brain stem; but no evidence has ever been presented which tended to show that stimulation at this level leads to disturbance of water metabolism. It is possible that fragments of the pituitary remained in the sella of the chronic cats. In two of them, in fact, tissue was found there at autopsy, though it was not histologically identified. But certainly in the acute animals no trace of any of the pituitary or hypothalamus remained. The chronic and acute animals taken together seem to indicate that the simple removal of hypophysis and hypothalamus can cause diabetes insipidus.

There can be no doubt that in the present experiments polyuria, when it appeared, was primary, because the animals' daily intake was limited to 200 cc. of milk.

The low chloride output of the group 2 cats, though inexplicable, requires comment. In eight of the nine cases, the trends of water and chloride excretion were roughly parallel to each other, as they are in graphs C and D of figure 2. Nevertheless, water and chloride excretion seem to be independent quantities, since this group, which surpassed all others in water output, eliminated an average of only 97 mgm. NaCl per day at a concentration usually below 0.1 per cent. Indeed the low average of chloride output combined with the unusually high volume output must imply an increasing chloride concentration within the animals. Such an accumulation might be sufficient reason for their short survival. We attempted to test the inference of accumulation by analysis of blood samples, but too few samples were obtained at the desired times to substantiate the point. Failure to eliminate chloride may have been due to diminished ability of the kidney to excrete chloride. However, in cases where chloride output improved progressively (for instance, the case of fig. 2 D), a corresponding improvement in kidney function is not necessarily indicated, since the blood chloride level was presumably increasing.

The term "decerebrate" is often used, as we have used it, in a broad sense which covers a rather wide variety of sections. Probably the commonest preparation described by this term corresponds closely to our animals of group 2. The point seems worth noting, since "decerebrate" animals have been used in several studies of water diuresis. Unless their

background of natural urine excretion is also studied, and found normal, it is not safe to extend conclusions obtained from such preparations to intact animals.

Group 3. Island section. The pituitary and the hypothalamus in the animals of group 3 were isolated nervously from the rest of the body by a vertical transection at level 2; but they were left in place with their blood supply as little damaged as possible. In eight cats the whole severed brain was left in place after the method described by Keller (1932). In the remaining twenty-two the first section, at level 2, was followed by another above it at level 1, and everything anterior to level 1 was removed (Bazett and Penfield, 1922). For our purpose, the latter method was better adapted: because, though the upper section may sometimes have invaded hypothalamic territory, or have wrought more disturbance to the circulation than Keller's method, it made the crucial lower section far easier to complete with certainty.

Both group 3 with this island section operation, and group 1 were intended to show whether the presence of the pituitary and hypothalamus could prevent polyuria. The island section was expected, besides, to test the humoral theory. If the polyuria which developed in eight out of nine cases in group 2 failed to put in its appearance in group 3, it would furnish strong evidence for the chemical nature of the influence upon water balance.

Actually, the volume output of island section cats averaged 97 cc. compared with 131 cc. for group 2. Individual figures give the same impression as group figures, that water excretion tends to be lower when the disconnected pituitary and hypothalamus are left in. The mean difference is rather large when one considers that limited intake must have levelled down the output of the animals with diabetes insipidus. Yet the variations, too, are large for a group which embraces only thirty individuals.

Two operative methods had been used in the preparation of group 3, but this fact does not explain the variability, since the results of both were equally divergent. Water excretion was completely independent of chloride excretion and of length of life. However, the group seemed to split quite sharply into two subgroups on the basis of survival time; or somewhat less clearly, on the basis of chloride output. These two factors ran roughly parallel. (See fig. 4.) Seventeen cats died in less than five days, like the animals of groups 1 and 2, and excreted 82 mgm. of NaCl per day. The rest lived an average of nine days and excreted 154 mgm. per day. The latter output, though still far from equalling their intake, is nearer normal than the output of either of the other groups. The long lived animals, moreover, appeared to be in water balance, since their weight losses were small, and their volume excretion lay between that of the control with water *ad lib.* and the controls on rigidly limited diet. In short, the island

section produced thirteen cats which were more stable and more nearly normal in excretion than any others of the operated cats. (See fig. 2 G, for an example.)

But it failed to do the like for the other seventeen. They are characterized in general by brief survival and a meagre chloride output. A few excreted chloride well; but at least a fraction of these may have been cats

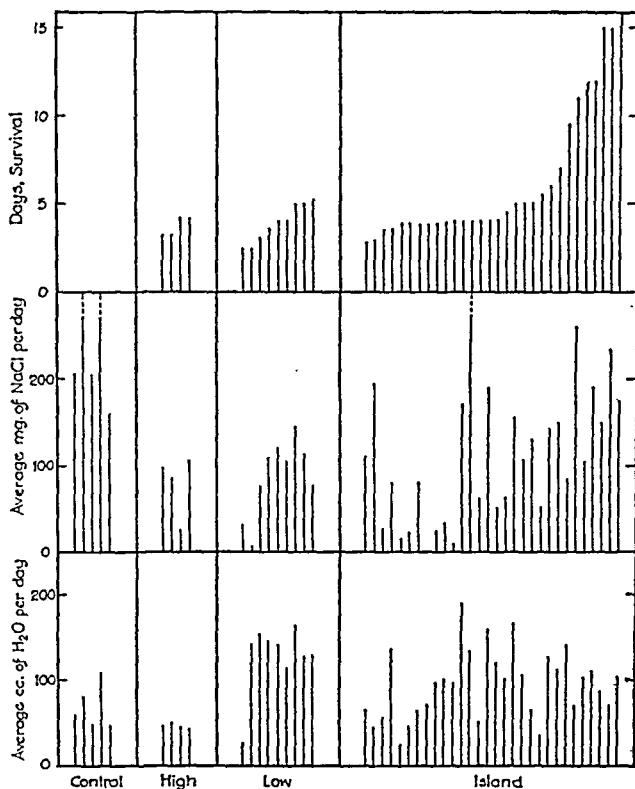


Fig. 4. Graph showing individual performance of both the controls and the chronic decerebrate animals. Ordinates are survival, average chloride, and average water output of single cats. Abscissae are the cats' numbers when they are arranged in order of increasing survival. Notice especially the clear division of group 3 into short and long survivals; and that a low chloride output usually corresponds to an incapacity to survive, but that water output bears no systematic relation either to survival or to chloride output. The limits of individual variation, though not of daily variation, appear in this graph.

destined to live long, and killed by mechanical accident. They fall naturally into still further subdivision on the basis of water excretion. Nine of them conveyed away their small output of salt in a small volume (fig. 2 E), as did group 1, but without any excessive muscular activity. The others approached the diabetes insipidus type of excretion, averaging around 100 cc. per day (fig. 2 F), and attaining, for one day each, the following maxima: 260, 260, 230, 190, 170, 160, 130, 130, 130 cc.

In group 3 the results, superficially considered, favor the humoral theory. Analyzed they are less convincing, but still suggestive in the same direction. The weak points in the evidence are two: first, that nine of the island section preparations developed polyuria, though the pituitary and hypothalamus were present; second, that in those animals which did not, the completeness of the lower transection was not demonstrated. With regard to the first difficulty, the impossibility of evaluating the importance of incidental damage wrought by the operation should be pointed out. When the double section was used, the upper one may sometimes have sloped so low as to damage nuclei essential for normal water balance. If control over water balance is normally exercised through a pituitary hormone, interference with its distribution would be virtually equivalent to ablation of the pituitary body. The upper section certainly crossed the third ventricle, and may have left it gaping widely. It may have altered the circulatory rate in the pituitary or hypothalamus. By either Keller's or Bazett and Penfield's method, a clot could have obstructed the aqueduct. Each of these contingencies interrupts one of the routes which have been suggested as possible avenues for the chemical influence from the pituitary. Thus there are many reasons why the essential structures, though still remaining, might be functionally useless. Under these conditions positive results,—the twenty-one cases in which the pituitary region did seem to prevent polyuria,—seem more convincing than the negative nine.

As for the completeness of the lower transection, this was admittedly problematical. We have excluded from the report those few cats in which autopsy, or the partial development of self temperature control, demonstrated its incompleteness. That completeness was crucial for the value of the experiment was constantly borne in mind at operation. We estimate that the number of cases in which it failed was relatively small.

The conclusion from the island section is thus drawn with less assurance than the two earlier conclusions. In thirteen cases the mere presence of the isolated pituitary and hypothalamus did prevent polyuria. It also seemed to make possible adequate chloride excretion and a fairly stable condition of health. Nevertheless, in seventeen other cases, life was short and chloride output low. Nine of these showed peaks of polyuria as high as did those cats which lacked pituitary and hypothalamus. A humoral control is compatible with the results in group 3, but not proved by them.

SUMMARY

Without attempting to solve the localization problem, we have studied the function of the pituitary and hypothalamus in regulating water and chloride excretion in chronic decerebrate cats, which were given 200 cc. of milk a day.

1. When the decerebration was high, so that the pituitary and hypothalamus remained, urine and chloride excretion were small, and life was short; but the significance of this result is complicated by the extreme muscular activity of this type of preparation.

2. When the decerebration was low, urine excretion was very large, but chloride excretion and length of life were about as small as in group 1. It is concluded that the polyuria which results in these cats is due to the removal of the pituitary and adjacent structures rather than to any irritation; and that polydipsia can play no part in the origin of this polyuria.

3. When the decerebration was low, but the pituitary and hypothalamus were left in place as an island of nervous tissue, some animals survived a considerable time, and approached the normal in water and chloride excretion. The remainder varied between the limits of group 1 and group 2. Equivocal evidence for the humoral theory is furnished by these results.

It is suggested that survival in decerebrate animals may depend in part upon their ability to keep themselves in chloride balance, and this in turn upon the intactness of the hypophysis and hypothalamus.

This investigation was aided by a grant from the Faculty Research Fund of the University of Pennsylvania.

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STUDIES ON WATER METABOLISM IN NORMAL AND HYPOPHYSECTOMIZED FROGS

MILDRED E. JONES AND F. R. STEGGERDA

From the Department of Physiology, University of Illinois, Urbana

Received for publication April 1, 1935

The function of the pituitary gland in regulating the melanophore reactions has been extensively studied and adequately reviewed by Hogben and Slome (1), Parker (2), and others. It has also been shown by Heller (3), Novelli (4), Dietel (5), Steggerda (6), and Steggerda and Essex (7) that when frogs are injected with posterior pituitary products, they increase markedly in weight due to uptake of water through the skin. Little is yet known, however, about the exact nature of this increased permeability of the skin resulting from the injections. This led to an investigation to determine if the pituitary gland might also govern the mechanism for the uptake of water following injections of pituitrin. The problem was studied by comparing the weight changes in normal and hypophysectomized frogs after pituitary injections.

The operation for removal of the hypophysis was similar to that described by Hogben (8), with this difference, that the gland was burned out instead of being removed by suction. All of the frogs showed the paleness characteristic of hypophysectomy. The criterion for the successful removal of the gland was that the frogs should remain pale on a dark background and should show no difference in body weight from the control animals over a period of 3 to 4 days. In certain cases where the frogs showed symptoms of brain injury, there was a marked increase in weight for a few days following the operation, after which time they died.

After enough frogs had been satisfactorily hypophysectomized, experiments were carried out to compare the effects of pituitrin (Parke-Davis) extracts on weight changes in normal and hypophysectomized frogs. Since these experiments were concerned with water interchanges as measured by weight, the frogs were kept nearly submerged in tap water for at least 10 to 12 hours before each experiment. At the time of the experiment, the frogs were removed separately from the water, dried with gauze as uniformly as possible, and weighed on a beam balance accurate to 0.1 gram. Then 11 hypophysectomized frogs (operated from 1-4 weeks previously) and 10 normal frogs were injected with obstetrical pituitrin, the dose being 0.1 cc. per 10 grams of frog weight. After the injections

all the frogs were replaced in the water and weighed at hour intervals for a period of six hours.

The results of these experiments show that when frogs hypophysectomized one to four weeks previous to the experiment are injected with the average dose of pituitrin, they show no sign of weight change other than that of uninjected normal control frogs. On the other hand, when normal frogs in the same water with the hypophysectomized frogs are injected with the same dose of pituitrin, they increase more than 18 per cent within

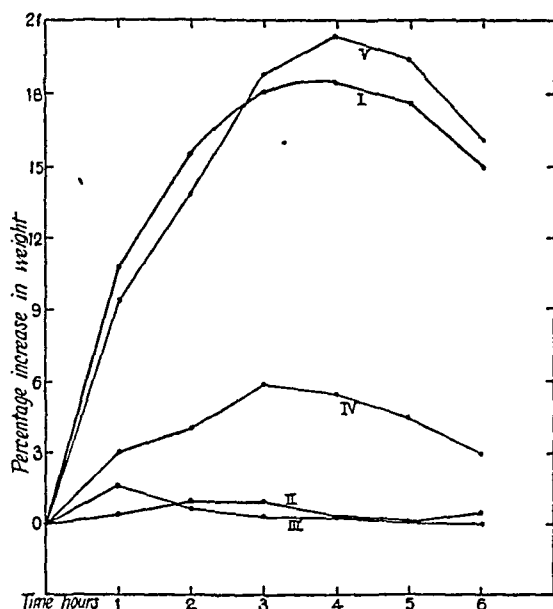


Fig. 1. Weight changes in normal and hypophysectomized frogs following injections of pituitrin.

Curve I. Average weight change of 10 normal frogs after injection of pituitrin.

Curve II. Average weight change of 11 hypophysectomized frogs injected with pituitrin (1-4 weeks after operation).

Curve III. Average weight change of 8 normal uninjected frogs.

Curve IV. Average weight change of 10 hypophysectomized frogs injected with pituitrin (1-4 days after operation).

Curve V. Average weight change of 5 frogs with cerebral hemispheres injured proximal to the pituitary gland, injected with pituitrin.

Note: The dose of pituitrin in all cases was 0.1 cc. per 10 grams of body weight.

four hours, and then gradually return to normal weight. This fact, we feel, clearly indicates that the presence of the pituitary gland in the frog is necessary in order that the pituitrin may cause some change in the skin which allows a greater absorption of water.

These results led us to inquire into the effects of pituitrin injections on weight changes in frogs shortly after the operation. A series of 10 frogs

hypophysectomized 1 to 4 days previously were injected with pituitrin (0.1 cc. per 10 gm. wt.). The average curve for this experiment indicates a slight but definite increase in weight, 6 per cent within three hours, and a gradual return to normal. This might mean that there are still some vestiges of pituitary products in the body of the frog, which continue to function in controlling the uptake of water after pituitary injections. This finding is quite in agreement with those of Heller (3) and Steggerda and Freedman (9), who report that the ability of frogs to respond to pituitrin is decreased after decapitation.

That the effects obtained are related to the removal of the pituitary gland, and not to injury of certain parts of the brain, was shown by experiments in which the cerebral hemispheres were injured just anterior to the hypophyseal region, and by others in which the brain had been destroyed with a pithing needle in the usual way. Although it is possible that pithing may injure the pituitary gland, the effects are not significant because when these frogs are injected with pituitrin, they show increases in weight very similar to those of normal frogs injected with pituitrin.

Each of the four experiments described was controlled by two normal uninjected frogs kept in the same water and weighed at the same time as the experimental frogs. Thus the control curve represents an average of eight frogs.

The question arose as to whether or not the dose used in the hypophysectomized animal was concentrated enough. This was tested by giving a double dose of pituitrin to 4 frogs hypophysectomized 2 to 4 days previously and to 3 normal frogs. It was found that the hypophysectomized animals showed no additional change in weight, whereas the normal frogs increased 32 per cent in body weight, nearly double the increase attained after a single dose. Repetition of a single dose of pituitrin to the same group of hypophysectomized frogs on two successive days was without effect, indicating the absence of any summation effects.

CONCLUSIONS

1. Hypophysectomized frogs, 1 to 4 weeks after operation, show no weight increase when injected with pituitrin. Control frogs similarly injected increase 18 per cent.

2. Hypophysectomized frogs, when injected with pituitrin 1 to 4 days after operation, show a slight increase in weight.

3. Cerebral injury does not alter the ability of pituitrin to produce weight changes.

4. The presence of the pituitary gland in the frog appears to be essential for the pituitrin to bring about the characteristic increase.

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THE PERMEABILITY OF FROG CAPILLARIES TO PROTEIN

RUTH E. CONKLIN

From the Department of Physiology, Vassar College, Poughkeepsie, N. Y.

Received for publication April 17, 1935

The marked permeability to protein of the capillaries of the frog's skin was first demonstrated by Churchill, Nakazawa and Drinker (1927). The plasma proteins are all found in the lymph from subcutaneous sacs, and other colloidal substances such as human hemoglobin and certain dyes pass easily through the capillary endothelium (Conklin, 1930b). Previous experiments, while they showed ease of passage from blood to lymph, indicated that normally this permeability was a one-way phenomenon, and that the protein, once out of the blood, must return to it by the lymphatic route. This left open the question whether the endothelial wall really prevented the return of protein to the blood, or whether it was largely a matter of a diffusion gradient. If, for instance, the concentration of protein in the blood as compared with the lymph could be reversed, the protein might then enter the blood through the capillary endothelium instead of by the circuitous lymphatic route.

METHOD. To test this hypothesis two series of experiments were undertaken in which the same method was followed as in earlier experiments, i.e., the washing out of plasma proteins by the injection of Ringer's solution into the ventral abdominal vein of curarized frogs, and the collection of lymph from drainage cannulas placed under the skin. Since curare stops the lymph hearts it would be impossible for protein in the lymph to return to the blood by the usual passage through the lymph hearts.

Series A. With the blood reduced in protein, as shown by refractometric determinations of the plasma and lymph, subcutaneous injections were made of frog plasma or normal horse serum. Three hours later a blood sample was taken from the heart and the plasma proteins determined refractometrically.

Series B. The serological method was used to detect the presence of horse serum in the blood. Horse-immune rabbit serum giving a titer of 1:16,000 was prepared for this purpose. A number of experiments were done first with *Rana pipiens*, but many were unsuccessful because of the difficulty of obtaining enough blood at the end of the experiment for a precipitin test. Additional experiments were, therefore, carried out on *Rana catesbiana*.

TABLE 1

EXPERIMENT NUMBER	LYMPH, PER CENT PROTEIN DURING BLOOD DEPLETION				INJECTION		PLASMA, PER CENT PROTEIN	
	$\frac{1}{2}$ hr.	1 hr.	1 $\frac{1}{2}$ hr.	2 hrs.	Amount	Per cent protein	Before injection	After injection
1	2.25		2.47	1.92	1 cc. horse serum	5.96	1.30	1.80
2	3.27		2.03		1 cc. horse serum	5.96	1.74	2.74
3		2.23	1.58		1 cc. horse serum	5.96	0.90	2.06
4	3.77	3.71	2.61	1.85	1 cc. frog plasma	3.94	0.23	2.34
5	4.21	3.39	2.74	2.05	1 cc. frog plasma	3.72	0.63	1.84
6	3.61	3.53	2.65	2.05	1 cc. frog plasma	3.94	0.49	1.60
7	4.21	3.55	2.96	2.39	None, control		0.97	2.48
8	0.83	1.06	0.94	0.83	None, control		0.45	0.69

TABLE 2

Frog serum tests with horse-immune rabbit serum

EXPERIMENT NUMBER	SPECIES AND TREATMENT	PRECIPITIN TEST AFTER 1 HOUR						
		Undiluted frog serum	1/10	1/100	1/500	1/1000	1/2000	1/4000
	<i>Experimental</i>							
10	{ R. pipiens Curare 10 cc. Ringer's solution 1 cc. horse serum }	++	+	-	-	-	-	-
11		++	+	-	-	-	-	-
12		+++	++	+	-	-	-	-
13		++	+	-	-	-	-	-
14	{ R. catesbiana Curare 100 cc. Ringer's solution 10 cc. horse serum }	+++	++	+	-	-	-	-
15		++	+	-	-	-	-	-
16		+++	++	+	±	-	-	-
17		+++	++	+	-	-	-	-
18		++	+	-	-	-	-	-
19		+++	++	++	+	-	-	-
	<i>Controls</i>							
1	R. pipiens (2)	-	-	-	-	-	-	-
	R. catesbiana	-	-	-	-	-	-	-
	No experimental procedure							
2	R. pipiens	+	±	-	-	-	-	-
	R. catesbiana	-	-	-	-	-	-	-
	Curare and horse serum; no blood depletion							
3	R. pipiens (2)	+++	++	+	-	-	-	-
	R. catesbiana	++++	+++	+++	++	+	-	-
	Horse serum only. No curare nor blood depletion							

The procedure consisted in curarizing the frog, then cannulating the ventral abdominal vein and injecting through it oxygenated Ringer's solution. In *Rana pipiens*, 10 cc. were injected over a period of two hours. In *Rana catesbiana*, which weighs approximately ten times as much as the leopard frog, 100 cc. were injected over the same length of time. Several cannulas were tied under the skin and lymph was drained from them. It is easy by this method to wash much of the protein out of the blood with only a slight rise in blood pressure (Conklin, 1930b), and there is the distinct advantage of avoiding withdrawal of blood as in plasmapheresis, a procedure very difficult in frogs owing to their small blood volume.

At the end of the injection period normal horse serum (1 cc. in *R. pipiens*, 10 cc. in *R. catesbiana*) was injected subcutaneously in the thighs, and the frog was left for a period of one, or usually two hours. A sample of blood was then withdrawn from the left aorta, allowed to clot, and the serum tested with horse-immune rabbit serum.

RESULTS AND DISCUSSION. *Series A.* The results are given in table 1. It will be seen that there was always an increase in plasma protein, indicating probable absorption through the capillaries. Since the control frogs, which were given no injection, also showed an increase, the method was not decisive in proving the added protein to have entered from the lymph sacs. Regeneration may have occurred very rapidly from the liver or elsewhere.

Series B. The serological findings are summarized in table 2. In all cases horse serum was found in the blood of curarized frogs. An inspection at the end of the experiment always showed that the lymph hearts were not beating, so that the horse serum must have entered the blood through the capillary endothelium. In the control experiments it is evident that in curarized frogs whose proteins have not been depleted the horse serum gets through the capillaries in very small amounts if at all, whereas in non-curarized frogs it can enter by the usual lymphatic route.

It seems evident that the normal, one-way permeability to protein of the skin vessels of the frog is due to a diffusion gradient rather than to any obstacle imposed by the endothelial wall. This same condition has been demonstrated in the dog by Field and Drinker (1931). Due to the enormously greater production of lymph in the frog than in mammals (Isayama, 1924a and b; Conklin 1930a), and to the permanent excess of capillary blood pressure over the osmotic pressure of the plasma colloids (Churchill, Nakazawa and Drinker, 1927) creating a steady hydrostatic flow outwards, there is even less chance in the frog than in mammals for reverse permeability to proteins under ordinary conditions. In spite of these factors it is plain that there can be passage of proteins into the capillaries, when the plasma protein content is depleted.

SUMMARY

1. The capillaries of the frog's skin, though highly permeable to protein, normally do not admit protein molecules from the lymph spaces.

2. When the plasma proteins have been depleted, horse serum, injected into subcutaneous lymph spaces in curarized frogs, will pass through the capillary endothelium and may be recognized in the blood by serological means.

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THE AMERICAN JOURNAL OF PHYSIOLOGY

VOL. 112

JULY 1, 1935

No. 3

THE EXCRETION OF INULIN BY THE DOG

JAMES A. SHANNON¹

*From the Department of Physiology, New York University College of Medicine,
New York City*

Received for publication February 27, 1935

The present paper reports observations on the excretion of the polysaccharid inulin by the normal and phlorizinized dog, these observations having been made prior to, or concurrently with, the observations on man recently reported by Shannon and Smith (1935). The introduction of inulin in renal function studies has been discussed by Richards, Westfall and Bott (1934), by Shannon (1934), by Professor Richards in his 1935 Harvey Lecture, and by Shannon and Smith in the above paper, and this discussion need not be extended here.

CHEMICAL METHODS. The chemical methods used in this investigation were the same as those described by Shannon and Smith (1935), except that in some of the earlier experiments sugars were determined both on a copper sulphate-sodium tungstate filtrate (Somogyi, 1931) by the Folin (1929) sugar method, as well as on the iron filtrate of Steiner, Urban and West (1932) by the Shaffer-Somogyi (1932) method. Extensive observations on recovery of inulin, glucose and xylose from plasma and urine have been made with both methods. The Folin method gives incomplete reduction with xylose but when plasma and urine are handled alike one should obtain the same U/P ratio by this as by the Shaffer-Somogyi method, especially since the saccharoid blank is low with both methods of precipitation (2 to 3 mgm. per cent with the copper and 1 to 2 mgm. per cent with the iron). Nevertheless, in experiments where both methods have been used, it has been observed that the Shaffer-Somogyi method gave values for the xylose clearance averaging five per cent lower than by the Folin method, although the inulin and glucose clearances by the two methods were identical. The reason for this discrepancy is not revealed

¹ This paper is based on a thesis to be presented to the Graduate School of New York University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

by recoveries. All data recorded here were obtained by the Shaffer-Somogyi method on an iron filtrate. White and Monaghan (1933), using this technique, were unable to obtain the nearly exact correspondences between the creatinine, glucose and xylose clearances previously reported from this laboratory for the phlorizinized dog. We have been unable to confirm these investigators, however, and continue to secure this correspondence, except as noted above.

EXPERIMENTAL PROCEDURE. Normal female dogs weighing from 15 to 18 kgm. were used in all our experiments. A 20 per cent solution of inulin in 0.6 per cent saline, prepared at 85°C., was administered either intravenously, subcutaneously, or by both routes. One does not obtain by the subcutaneous route plasma concentrations higher than 100 mgm. per cent even when fairly large doses are given, and for this reason the inulin was given in most of our experiments intravenously in doses of from 2 to 4

TABLE 1

Showing the relationship existing between plasma level and rate of excretion of inulin

DOG D			DOG 8		
Plasma	Urine	Clearance	Plasma	Urine	Clearance
<i>mgm. per cent</i>	<i>mgm. per min.</i>	<i>cc. per min.</i>	<i>mgm. per cent</i>	<i>mgm. per min.</i>	<i>cc. per min.</i>
565	350	62.0	309	108.8	35.2
380	237	62.4	227	82.6	36.4
230	139	60.4	164	59.2	36.1
143.5	92.2	64.2	120	42.0	35.0
86	53.3	62.0	88.2	32.0	36.2
53.5	32.8	61.4	63.8	22.8	35.8

grams per kilogram, or in doses of from 1 to 2 grams per kilogram subcutaneously followed by a like amount intravenously.

Xylose was administered by stomach, creatinine subcutaneously, and phlorizin intravenously or by a combination of subcutaneous and intravenous injections. The conduct of the experiments was on the whole identical with those previously reported.

RESULTS. The experiments reported in table 1 show that the rate of excretion of inulin in the dog is directly proportional to the plasma concentration between values of 53 and 565 mgm. per cent. It follows from this fact that the inulin clearance is independent of its plasma level. The curves generated by plotting plasma level against rate of excretion extrapolate through the zero coördinates, indicating that the relationship holds true at very low as well as at the observed plasma levels.

An experiment showing that the administration of inulin intravenously does not affect the clearances of urea, xylose or creatinine, either absolutely

Data to show that the intravenous infusion of inulin has no effect on the renal clearances of other substances

PERIOD NUMBER	URINE FLOW	PLASMA				CLEARANCES				CLEARANCE RATIOS					
		Urea	Xylose	Inulin	Creatinine	Urea	Xylose	Inulin	Creatinine	$\frac{\text{Urea}}{\text{Xylose}}$	$\frac{\text{Creatinine}}{\text{Xylose}}$	$\frac{\text{Urea}}{\text{Inulin}}$	$\frac{\text{Xylose}}{\text{Inulin}}$	$\frac{\text{Creatinine}}{\text{Inulin}}$	
	cc. per min.	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	cc. per min.	cc. per min.	cc. per min.	cc. per min.						
1	2.53	12.4	80.5		24.6	21.9	29.5		41.1	0.74	1.39				
2	2.10	12.5	80.7		27.6	19.0	25.4		38.0	0.75	1.50				
3*	1.83	12.6	81.7		29.4	19.1	26.0		38.6	0.74	1.48				
Average.....						20.0	27.0		39.2	0.74	1.46				
4	2.00	12.6	81.8	139	37.0	18.4	23.4	35.0	34.4	0.78	1.47	0.53	0.67	0.98	
5	1.53	12.9	77.6	101	37.4	20.8	28.5	41.6	40.9	0.72	1.44	0.50	0.69	0.98	
6	0.88	13.3	72.6	78	36.8	20.6	28.6	41.5	41.3	0.72	1.44	0.50	0.69	1.00	
Average.....						19.9	26.8	39.4	38.8	0.74	1.45	0.51	0.68	0.99	

PERIOD NUMBER	URINE FLOW	PLASMA			CLEARANCE			CLEARANCE RATIOS	
		Xyloso	Inulin	Creatininio	Xyloso	Inulin	Creatininio	Xyloso Inulin	Creatininio Inulin
	cc. per min.	mgm. per cent	mgm. per cent	mgm. per cent	cc. per min.	cc. per min.	cc. per min.		
1	3.6	50.3	104	13.7	35.0	48.6	49.1	0.72	1.01
2	3.33	51.6	106	14.7	34.8	47.7	49.1	0.71	1.03
3	3.13	53.1	108	16.0	36.0	50.7	50.1	0.72	0.99
4	2.87	54.0	109	16.6	33.9	47.1	47.6	0.72	1.01
Average.....								0.72	1.01

or relative to each other, is given in table 2. This point is an essential one in the administration of any substance for renal function studies.

The creatinine clearance is identical with that of inulin under all conditions that we have examined. A typical experiment illustrating simultaneous creatinine and inulin clearances is given in table 3, and data from the entire series are given in figure 1. The mean of 42 comparisons on 8 dogs gives a creatinine/inulin ratio of 0.994 with a standard deviation of 0.034, and maximum variations of $+0.086$ and -0.064 . In these experi-

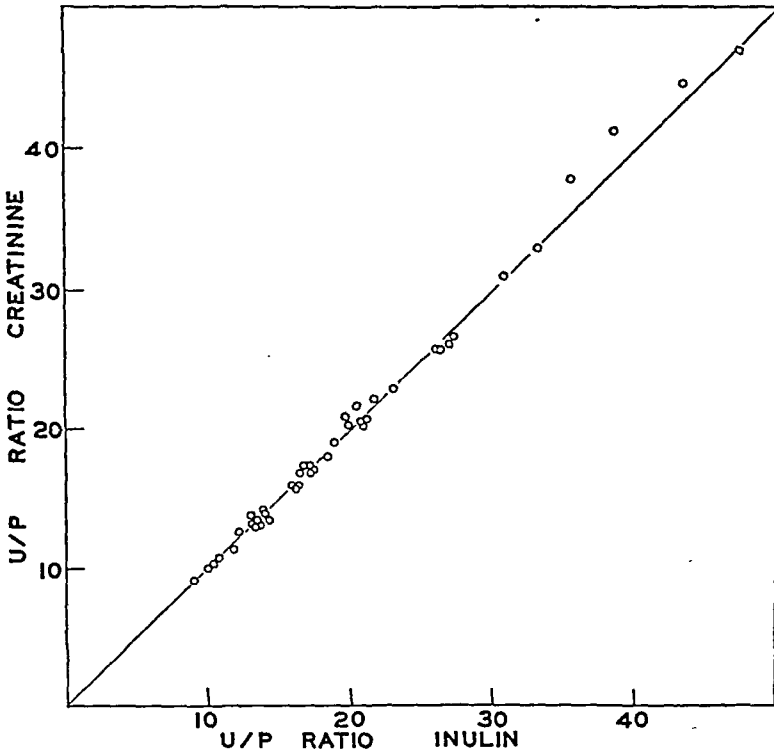


Fig. 1. Data showing that the simultaneous U/P ratios (and therefore the clearances) of creatinine and inulin are equal in the normal dog. This equality is maintained after phlorizin.

ments the plasma creatinine varied from 6.0 to 130 mgm. per cent, and the U/P ratio of inulin from 9 to 47. Lack of space precludes the presentation of the data in full, but it may be commented that we find the creatinine clearance to be independent of the plasma level of this substance, as is the case with inulin.

The xylose clearance in the normal dog is less than the simultaneous inulin clearance. In this finding we confirm Richards, Westfall and Bott (1934), whose data, however, show a wider deviation between the two clearances than ours do. A series of 24 observations on 6 dogs is illustrated in figure 2. The mean xylose/inulin ratio in these data is 0.734

and the standard deviation of the mean is 0.022. All these observations were made at urine flows above 0.88 cc. per minute, but with inulin U/P ratios ranging from 11.1 to 76.2. A larger series of observations on this point was not considered necessary since the creatinine clearance has been shown in a number of previous papers to exceed the xylose clearance by about this amount.

Data from a typical experiment illustrating the action of phlorizin are given in table 4, and a summary of observations of 5 dogs before and after phlorizin (30 periods in all) is given in table 5. Since phlorizin depresses all renal clearances it is necessary in making comparisons before and after the administration of this drug to take one clearance as a standard of refer-

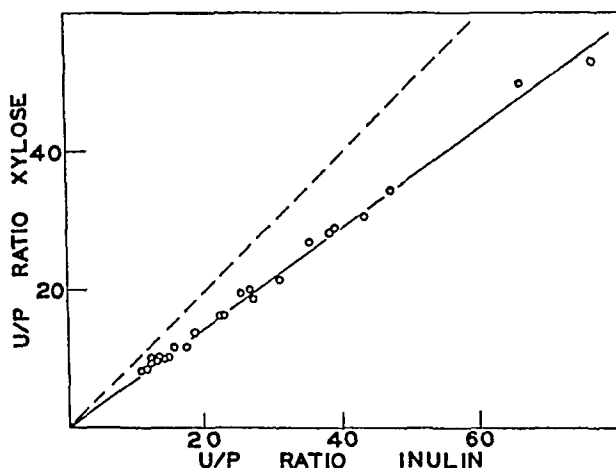


Fig. 2. Data showing that the U/P ratio (and therefore the clearances) of xylose in the normal dog is less than the simultaneous U/P ratio of inulin. The dotted line represents a xylose/inulin ratio of 1.0, and the solid line a ratio of 0.734, the mean of the observations. Phlorizin raises the xylose clearance to within 10 per cent of the inulin clearance.

ence. In tables 4 and 5 we have chosen the inulin clearance for this purpose. The administration of phlorizin has no effect upon the relative values of simultaneous inulin and creatinine clearances, these remaining identical within the limits of experimental error. Glucose, of course, appears in the urine after phlorizin, the clearance of this substance being identical with that of creatinine, as previously reported by Shannon, Jolliffe and Smith (1932), and therefore identical also with the inulin clearance. After phlorizin the xylose clearance rises relative to the inulin clearance, but does not come to equal it, remaining about 10 per cent below the latter. (Comparisons made by the Folin sugar method on a copper tungstate filtrate give xylose clearances about 5 per cent higher, as reported under Methods; in view of this fact the xylose clearance may really be within 5 per cent of the inulin clearance in the phlorizinized ani-

TABLE 4

Comparison of urea, xylose, glucose, inulin and creatinine clearances before and after phlorizin (100 mgm. per kgm. intravenously)

Dog 8. February 15, 1934.

PERIOD NUMBER	URINE FLOW	PLASMA					CLEARANCE					CLEARANCE RATIOS			
		Urea	Xylose	Glucose	Inulin	Creatinino	Urea	Xylose	Glucose	Inulin	Creatinino	Urea Inulin	Xylose Inulin	Glucose Inulin	Creatinino Inulin
	cc. per min.	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	cc. per min.	cc. per min.	cc. per min.	cc. per min.	cc. per min.				
1	2.30	11.5	75.0		174	30	20.1	27.3		37.2	36.6	0.54	0.73		0.98
2	1.65	11.6	73.4		139	32	19.8	26.9		38.0	38.0	0.52	0.71		1.00
3	1.20	11.7	66.8		114	33.5	19.8	25.9		36.7	37.2	0.54	0.71		1.01
Average.												0.53	0.72		1.00
4*	1.20	11.7	54.0	147	86.7	35.9	15.2	23.2		25.0	24.5	0.61	0.93		0.98
5	1.20	11.7	49.0	139	79.3	34.9	14.4	22.0	24.8	25.0	24.5	0.58	0.88	0.99	0.98
6	1.14	11.7	41.6	131	67.0	34.1	14.8	22.8	26.6	26.0	26.0	0.57	0.88	1.02	1.00
Average.												0.59	0.90	1.01	0.99

* Following Phlorizin, 100 mgm. per kilogram, intravenously.

TABLE 5

Summary of comparisons of urea, xylose, inulin, creatinine and glucose clearances before and after phlorizin

ANIMAL NUMBER	BEFORE PHLORIZIN					AFTER PHLORIZIN							
	Number of periods	Inulin clearance*	Clearance ratios			Dose of phlorizin		Number of periods	Change in inulin clearance*	Ratio of clearances*			
			Urea Inulin	Xylose Inulin	Creatinine Inulin	Intra- venously	Subcu- taneously			Urea Inulin	Xylose Inulin	Creatinine Inulin	Glucose Inulin
		cc. per min.				mgm. per kilo	mgm. per kilo		per cent				
4	3	35.5			0.98	200		3	-19.0			0.98	0.97
5	3	46.6			0.99	100	100	3	-14.0			0.99	0.99
7	3	72.2		0.77	0.99	100	100	3	-44.0		0.93	0.96	1.07
8	3	37.3	0.53	0.72	1.00	100		3	-31.0	0.59	0.90	0.99	1.01
30	3	75.7	0.63	0.76	1.06	100		3	-31.0	0.64	0.90	1.02	1.02
Average.....			0.58	0.75	1.00				-28.0	0.61	0.91	0.98	1.01

* Average of all periods.

mal.) In a limited series of experiments no significant change has been observed in the urea/inulin ratio before and after phlorizin.

The excretion of sucrose relative to inulin has not been extensively examined. In those experiments where this substance was present the results were in accord with the relationship reported here between xylose and inulin.

DISCUSSION. The only independent evidence that can be advanced that inulin is not excreted in part by tubular secretion in the normal dog, is the fact that its clearance is independent of the concentration in the plasma, between wide limits of the latter. Since this evidence is not absolutely exclusive, the presumption against secretion must rest upon the evidence obtained from the aglomerular fish (Richards, Westfall and Bott, 1934; Shannon, 1934) and the behavior of carbohydrates in general, as discussed by Shannon and Smith (1935). It is significant that in the dog the creatinine clearance is independent of the plasma level of this substance, as has previously been pointed out from this laboratory, and confirmed in the experiments described in this paper. The behavior of this substance in the dog is quite different from what it is in man, where a markedly curvilinear relationship is observed (Shannon, 1935). Granted that a linear relationship between plasma level and rate of excretion is, in these two instances, evidence against secretion of either substance, it is a confirmation of this evidence that the simultaneous inulin and creatinine clearances are equal, within the experimental error of observation, in both the normal and phlorizinized dog.

It follows from the above interpretation regarding the non-secretion of inulin that some xylose is reabsorbed in the normal dog, the figures presented here indicating the fraction to be about 27 per cent of that which is filtered. The fact that the xylose/inulin ratio does not vary with variation in inulin U/P from 11.1 to 76.2 might be taken to indicate that the degree of concentration of the urine is not the predominating factor in the discrepancy between the excretion rates of these two substances.

Since phlorizin raises the xylose clearance relative to the inulin clearance, it must be inferred that reabsorption in the dog, as in the dogfish and man, is in part an active process. In the three experiments on the phlorizinized dog reported here, the xylose clearance is still 10 per cent below the inulin clearance, a difference that is only 5 per cent when the Folin method, instead of the Shaffer-Somogyi method, is used. In view of the much larger series of comparisons previously published from this laboratory (in which the Folin sugar method and copper tungstate filtrate were used), showing almost exact agreement in the clearances of these substances in the phlorizinized animal, the significance of this difference is questionable.

Shannon, Jolliffe and Smith (1932) based their conclusion that creati-

nine was secreted in the normal dog on the facts that: *a*, the creatinine clearance exceeds the simultaneous xylose and sucrose clearances; *b*, under phlorizin, these clearances come to equal each other, an equalization that is effected by a fall in creatinine clearance rather than a rise in xylose clearance; and *c*, phlorizin does not change the urea/xylose ratio, as might be expected if the drug were specifically blocking the reabsorption of the pentose. Pitts (1934) in a subsequent examination of the excretion of creatine, confirmed the first two observations, noting that on the administration of phlorizin, the creatine and creatinine clearances dropped to approximately the same level, the glucose rose to the xylose level, and the xylose clearance itself was not appreciably changed. The constancy of the xylose clearance before and after phlorizin may, however, be an artifact due to a decrease in glomerular filtration equal to a diminished reabsorption of the sugar. The fact that in Pitts' (1934) experiments the average creatine/creatinine ratio remained constant (0.89 before and 0.92 after phlorizin) in spite of a fall in the absolute value of both clearances, whereas the ratio of the xylose clearance to the other two rose, points in this direction. The apparent constancy of the urea/xylose ratio before and after phlorizin, which Jolliffe, Shannon and Smith (1932) emphasized as evidence against reabsorption of xylose, has also been noted in man (Chasis, Jolliffe and Smith, 1933) but, as Shannon and Smith (1935) have pointed out, this ratio is now known to change with changes in the absolute level of these clearances, and such independent changes may partly or wholly occlude the change due to the blockage of the reabsorption of the sugar.

It would appear from the evidence obtained by the use of insulin that xylose is actively reabsorbed in the normal dog, and that the difference between the xylose and creatinine clearances is due to this reabsorption. The extent of this reabsorption, as judged from the inulin clearance, appears to be about the same in the dog as in the dogfish and man.

When we consider all the evidence available on the dogfish, man, and dog, we are led to the following interpretation: that inulin is not secreted by any of these and, assuming no reabsorption of this substance (*vide infra*), its clearance is very close to, if not identical with, the rate of glomerular infiltration. The capacity to secrete creatinine, evident in the dogfish and man, is vestigial or absent in the dog: hence the equality of the inulin and creatinine clearances. This equality is not perturbed by phlorizin, and since phlorizin brings the glucose clearance up to the inulin and creatinine clearances, it appears that this drug completely blocks the reabsorption of glucose in the renal tubules, as was believed by Jolliffe, Shannon and Smith (1932).

Since an apparent active reabsorption of some xylose has been uncovered in these experiments, it seems possible that there might also be an active reabsorption of some inulin. No evidence bearing on this question can be

advanced at the present time, unless it be the mere fact of the identity of the creatinine and inulin clearances in the normal animal.

With regard to the passive reabsorption (diffusion) of inulin, it will be noted that the equality between the creatinine and inulin clearances holds at U/P ratios of inulin varying from 9 to 47, and that in the phlorizinized dog this equality also obtains with the simultaneous glucose clearance. It has been shown that the simultaneous xylose and sucrose clearances in the normal dog, and xylose and glucose clearances in the phlorizinized dog, are equal (Jolliffe, Shannon and Smith, 1932; Pitts, 1934); it appears from these facts that the differential diffusion of these substances must be very small, and that the diffusion of a molecule as large as inulin (mol. wgt. 972 or greater) is probably negligible. But this argument cannot be extended to the abnormal kidney, or to the normal kidney at very low urine flows, without the support of further evidence.

SUMMARY

The rate of inulin excretion in the dog is directly proportional to plasma concentration between values of 53 and 565 mgm. per cent.

The intravenous administration of inulin does not affect the urea, xylose or creatinine clearances.

In a series of 42 comparisons on 8 dogs, the ratio of the simultaneous creatinine and inulin clearances has a mean value of 0.994 with a standard deviation of 0.034, and maximum variations of $+0.086$ and -0.064 .

The xylose clearance in the normal dog at moderate to high urine flows is less than the simultaneous inulin clearance, the mean xylose/inulin ratio being 0.734, with a standard deviation of 0.022.

Under phlorizin the glucose clearance rises to the level of the creatinine and inulin clearances, the equality of which is maintained, while the xylose clearance is raised to within 10 per cent of the inulin clearance.

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THE EFFECT OF ONE BORDER IN THE VISUAL FIELD UPON THE THRESHOLD OF ANOTHER

GLENN A. FRY AND S. HOWARD BARTLEY¹

From the Laboratory of Neurophysiology, Washington University, St. Louis

Received for publication March 18, 1935

Two types of test objects are widely used for measuring brightness discrimination. One consists of a disk on a slightly darker field. In this case the capacity to discriminate brightness is measured by the minimum brightness difference between the area and its background which is just perceptible. This is called the brightness difference threshold. Although it is generally spoken of as the threshold of the area, it may be regarded as the threshold of the border, and this way of conceiving the threshold will be employed in the present paper.

The second type of test object used for measuring brightness discrimination is a bisected object, one half of which is slightly darker than the other. The threshold in this case is the brightness difference between the two halves necessary for the border between them to be just perceptible.

In the present paper we shall refer to the border for measuring the threshold as the *test* border. Other borders in the vicinity which affect the threshold will be called activating borders. In order to give the reader a clue for understanding and relating the numerous phenomena to be described, it may be pointed out here that whenever an activating border acts on the side of a test border, as must always be the case when the test border is continuous, the effect is invariably an interference with the establishment of the border which raises the threshold. Figure 2 presents a situation of this kind. When, as happens in the case of figure 7 where a bisected test-object is used, the activating border acts on the ends of the test border, it facilitates the establishment and thus lowers the threshold.

Although previous investigators have reported phenomena which are related to this problem, they have in some cases attributed the results to unessential factors in the stimulus conditions. In such cases it is necessary to analyze carefully the rôles played by the various factors. In the second place we have tried to tie together systematically a number of phenomena which have heretofore been treated in isolation from each other.

The first phenomenon to be considered was first described by Blachow-

¹ Beneficiary of a Grant-in-aid for Research in Neurophysiology from the Rockefeller Foundation.

ski (1913). He used a stimulus pattern similar to figure 2 and showed that the differential threshold between the disk, *A*, and the annulus, *B*, decreases as the width of the annulus, *B*, increases. The results we have obtained are shown in figure 4. Blachowski attributed his phenomenon to the inclusion of more area within *B*. The inadequacy of this factor to account for the phenomenon can be shown by using a stimulus pattern like figure 3. The width of the black band and the outside diameter of *C* were kept constant so that the total area ($C + B$) remained constant,² and the only factor varied was the distance of the black ring from *A*. The explanation of the decrease in threshold in this case seems to be that the border at the outer edge of *B* interferes with the establishment of the border at the edge of *A*, and the amount of interference decreases as the outer edge of *B* gets more and more remote from the edge of *A*. At a distance of $1\frac{7}{16}$ inches the interference becomes practically negligible. Since the same results are obtained with figure 2 as with figure 3, the decrease in the threshold may be attributed to a release from the interference effect of the outer edge of *B* rather than to the inclusion of more area within *B*.

The pattern in figure 3 demonstrates a second fundamental property of a border, that of blocking activity from spreading across the retina. Since the same results are obtained with the annulus, *C*, present and absent, it must be assumed that the border at the outer edge of *B* blocks the border at the inner edge of *C* from affecting the threshold of *A*.

Blachowski's phenomenon is closely related to the fact that the threshold for an area on a slightly darker ground decreases as the size of the area increases. Although previous investigators have attributed this to the inclusion of more receptors within the area, evidence is presented below that the essential stimulus factor is the separation of the borders. In other words when a disk shaped area is used for measuring the threshold, the borders on opposite sides of the area tend to interfere with each other, so that, when the disk is small, the interference is great and the threshold high, but this interference dies out as the separation of the borders increases, and consequently the threshold falls.

We have measured the differential threshold for disk-shaped areas subtending visual angles ranging from 0.43° to 8.05° . The results are shown in figure 5. When these results are compared with the results of the Blachowski experiment shown in figure 4, it is seen that change in threshold dies out at the same separation of borders in both cases, namely, about 4° . This may be interpreted as evidence that the variation of area experiment as well as Blachowski's experiment involves the interference of one border by another.

The same type of thing comes into play in Dittmers' (1929) experiment,

² As the diameter of the ring increases its area also increases, thus changing the total area of $C + B$, but the change is so slight that it may be ignored.

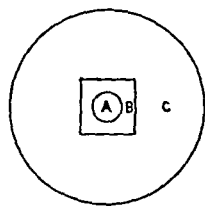


Fig. 1

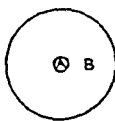


Fig. 2

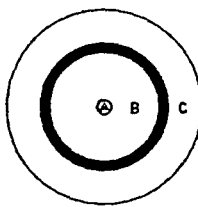


Fig. 3

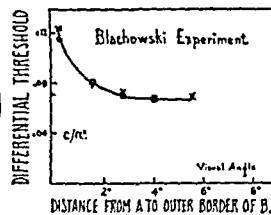


Fig. 4

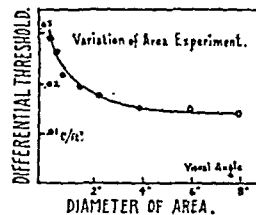


Fig. 5

Fig. 1. Pattern for demonstrating Dittmers' phenomena. C was $1\frac{1}{4}$ inches in diameter. B was $\frac{1}{2}$ inch on each side. A was $\frac{1}{4}$ inch in diameter. The distance from the stimulus to the eye was 38 inches. An artificial pupil 2.33 mm. in diameter was placed in front of the right eye. The left eye was shielded completely. The experiment was performed in a dark room with nothing visible but the stimulus pattern. The object of the experiment was to investigate the effect of varying the brightness of C upon the differential threshold for A .

Fig. 2. Pattern for demonstrating Blachowski's phenomenon. The diameter of A was $\frac{3}{8}$ inch. The brightness of B was set at 0.468 c. per sq. ft. An artificial pupil 2.33 mm. in diameter was placed before the right eye. The left eye was shielded completely. The stimulus was $28\frac{1}{2}$ inches from the eye. The object of the experiment was to demonstrate the effect of varying the size of B upon the differential threshold for A .

Fig. 3. Pattern for demonstrating the inadequacy of Blachowski's type of explanation. The diameter of A was $\frac{3}{8}$ inch. The outside diameter of C was 6 inches and the width of the black band was $\frac{1}{4}$ inch. The brightness of C and B was kept constant at 0.468 c. per sq. ft. Other conditions were the same as for the pattern in figure 2. The object of the experiment was to investigate the effect of varying the diameter of the black band upon the threshold for A , and to ascertain whether the presence of the annulus, C , has any effect upon the results as compared with the results obtained with the pattern in figure 2.

Fig. 4. An analysis of Blachowski's phenomenon as demonstrated with the patterns in figures 2 and 3. The crosses show the effect of varying the distance from A to the outer border of B in figure 2. The circles show the effect of varying the distance from A to the outer border of B in figure 3 with the width of the dark ring and the outside diameter of C kept constant. The differential threshold specifies the brightness difference between A and B necessary for A to be just perceptible. In these experiments A was always brighter than B . Fry served as subject.

A series of five measurements of the threshold for A was made for each size of B for both patterns. The series for each size of B for figure 2 was followed by a series for figure 3 with B of the same size. In this way readings for the two different patterns for a given size of B were made with the condition of the eye practically unchanged. The different sizes of B were investigated in the order of increasing size. The experiment was repeated in reverse order and the results were averaged. Hence each value in the graph in figure 4 represents an average of 10 readings. At the beginning of the experiment and between each pair of series dealing with a given size of B , a series of readings was made with figure 3 from which the black band was removed. This pattern served as a standard for measuring changes in the general condition of the eye. The threshold with this pattern remained practically unchanged during the course of the experiment, showing an average value of 0.07 c. per sq. ft.

Fig. 5. The effect upon the brightness difference threshold of varying the diameter of an area. Areas of different sizes were presented on a bright background (0.9 c. per sq. ft.) which was 16 inches by 20 inches and 20.75 inches from the eye. Two readings were made for each area, one series being made in the order of increasing size and the other in the order of decreasing size. Bartley served as subject, using one eye without an artificial pupil.

which can be demonstrated with the pattern in figure 1. The object of the experiment is to investigate the effect of varying the brightness of *C* upon the differential threshold for *A*. We have performed two separate experiments, one in which *A* was brighter than *B* and another in which *A* was darker than *B*. The results are shown in figure 6. In both cases the mini-

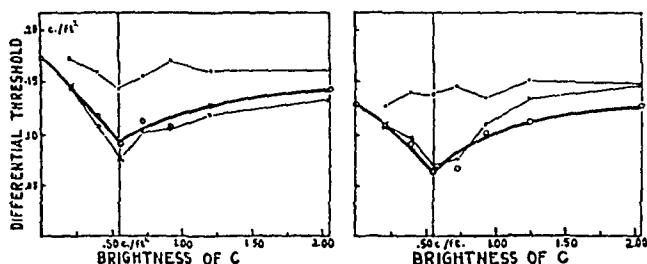


Fig. 6. These graphs show the results obtained with the stimulus pattern in figure 1. The object of the experiment was to investigate the effect of varying the brightness of *C* upon the differential threshold for *A*, i.e., the brightness difference between *A* and *B* necessary for *A* to be just perceptible. In the left hand graph *A* was brighter than *B*; *B* was kept constant at 0.565 c. per sq. ft. (the level indicated by the vertical line), and *A* was varied to measure the threshold. In the right hand graph *A* was darker than *B*; *A* was kept constant and *B* varied.

In each experiment the measurements were all made at one sitting. The different levels of brightness of the surrounding field were taken in the order of increasing brightness. Alternate readings were made with the surrounding field present and absent. Five such pairs of readings were made at each level. The values given in the graph represent therefore averages of five readings. When the readings were alternated with the surrounding field present and absent, changes in the condition of the eye could be detected, if any occurred during the experiment, by the change in the threshold with the surrounding field absent. The empirical values obtained with the surrounding field present can be corrected for changes in the condition of the eye by determining the ratio between the value for "field present" and "field absent" and multiplying the original value for "field absent" by this ratio. These corrected values give a truer expression of the phenomenon than the raw empirical data. In each graph the line through the dots represents the change in the general condition of the eye during the course of the experiment as measured by the differential threshold between *A* and *B* when *C* was zero. The crosses represent the absolute values obtained for the different brightness levels of *C*, and the circles represent the corrected values as explained above. Fry served as subject in these experiments.

mal threshold value was obtained when *B* and *C* were equal in brightness; the threshold rises when *C* is either higher or lower than *B*.

Dittmers suggested that contrast which maintains the border between *B* and *C* is the factor responsible for the behavior of the threshold, and in accordance with this explanation the interpretation of the salient facts may be summarized as follows: 1. When the surrounding field, *C*, is either darker or brighter than zone *B* the resulting contrast border interferes with the establishment of a border at the edge of *A* and hence raises the thresh-

old for *A*. 2. As the brightness of *B* and *C* approach equality, contrast between them is lessened, and the border of *A* is gradually released from interference, and as a result the threshold falls. 3. It does not matter which of the two sides of the activating border is dark or bright, it has the same qualitative effect upon the border, one of interference. 4. Nor does it matter whether *A* is darker or brighter than *B*, the effect upon the border between them is still one of interference. 5. Since the border between *A* and *B* is a circle, the activating border acts sidewise against it, because there is no end to be acted upon.

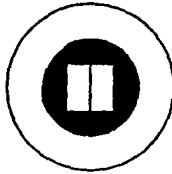


Fig. 7

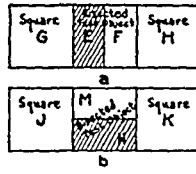


Fig. 8

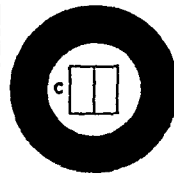


Fig. 9

Fig. 7. Pattern for demonstrating the effect of the dark side of an activating border upon the ends of a test border. The thickness of the ring was $\frac{3}{8}$ inch. The inside diameter was 1 inch. The bisected test object was $\frac{1}{2}$ inch on each side. The brightness of the dark half of the test object was set at 0.565 c. per sq. ft. and that of the bright half was varied to measure the threshold. The effect upon the threshold of varying the brightness of the ring was investigated. The distance to the eye was 38 inches. The left eye was shielded and an artificial pupil 2.33 mm. in diameter was placed before the right eye.

Fig. 8. Patterns for analyzing the effects of varying the brightness of *C* in figure 9. In each pattern the squares as well as the bisected test object were $\frac{1}{2}$ inch on each side. The brightness of the dark half of the test object was set at 0.565 per sq. ft. and the bright half was varied to measure the threshold. The effect upon the threshold of varying the brightness of the squares was investigated. The distance to the eye was 38 inches. The left eye was shielded and an artificial pupil 2.33 mm. in diameter was placed before the right eye.

Fig. 9. Pattern for demonstrating the effect of the bright side of an activating border upon the ends of a test border.

Geldard's phenomenon (Geldard, 1931; Bartley and Fry, 1934), demonstrates the effect of an activating border upon the end of a test border. The phenomenon can be demonstrated with a pattern like figure 7, although in Geldard's original pattern a bright disk to one side of the test object was used instead of a ring completely surrounding it. The inside border of the white ring acts upon the ends of the bisecting border, but is prevented from acting upon the sides of the bisecting border by the borders which separate the two halves of the test object from the surrounding field. It is impossible to demonstrate this by any manipulation of figure 7, but it is quite possible under more adequate circumstances to demonstrate that one border can block the activity spreading from another, and there seems

to be no reason why the same principles should not hold in the case in question. An instance of the blocking of the effects of one border by another is found in the case of figure 3, for the outer border of *B* blocks the inner border of *C* from affecting *A*.

The effect of varying the brightness of the ring in figure 7 upon the threshold of the bisecting border is shown in the left hand graph in figure 10. As the brightness of the ring increases the threshold of the bisecting border decreases. The interpretation of this is that when an activating border acts upon the ends of a test border, it lowers its threshold.

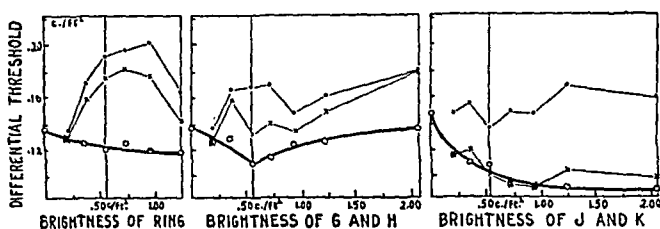


Fig. 10. The left hand graph shows the effect of the dark side of a border upon the end of a test border as demonstrated by varying the brightness of the ring in the pattern in figure 7.

The center graph shows the rôle played by borders at the sides of a bisected test object as demonstrated by varying the brightness of the two squares, *G* and *H*, in figure 8a.

The right hand graph shows the effect of the bright side of a border upon the ends of a test border as demonstrated by varying the brightness of *J* and *K* in the pattern in figure 8b.

The differential threshold specifies the difference in brightness between the two halves of the test object necessary for a perceptible border. The brightness level of the dark half of the test object is indicated in each graph by the vertical line. In each graph the line through the dots represents the change in the sensitivity of the eye during the course of the experiment as measured by the threshold for the test object alone in a dark field. The crosses represent the absolute values obtained for the different conditions, and the circles represent the corrected values as explained in the legend of figure 6. The same procedure as to order of readings has been used in these experiments as described in the legend of figure 6. Fry served as subject.

Figure 9 is strictly analogous to figure 7 but involves a reversal of brightness relationships. The pattern in figure 9 is the most difficult to deal with of any that we have yet considered. The effects obtained from varying the brightness of zone *C* are confusing, because this stimulus variable manipulates the borders both at the outer edge of *C* and at the juncture between *C* and the test object. It is highly desirable to demonstrate the effects of these borders separately and to this end the two patterns in figure 8 have been designed. In both patterns the effect of varying the brightness of the squares was investigated. The results are given in the center and the right hand graph in figure 10.

The experiment with figure 8a is simply a modification of Dittmers'

experiment. The effect of the border between G and E upon the bisecting border is analogous to the effect of the border between B and C in figure 1 upon the border at the edge of A when A is brighter than B . Likewise the effect of the border between H and F upon the bisecting border is analogous to the effect of the border between B and C in figure 1 upon the border at the edge of A when A is darker than B . Therefore on the basis of the results obtained with figure 1 we should expect that the borders between E and G and between F and H would interfere with the establishment of the bisecting border when G and H are above or below E and F in brightness, and that this interference would decrease as G and H approach the brightness level of E and F . This is exactly what happens.

The same type of phenomenon as that just described has been demonstrated in an experiment by Cobb (1916) who investigated the effect of varying the brightness of a field surrounding a bisected test object, but instead of using a field of limited size and definitely bounded like figure 9, Cobb included the whole field of vision. In this situation the only borders which can act upon the bisecting border are those which separate the two halves of test object from the surrounding field. These borders tend to raise the threshold for the bisecting order, and hence the threshold should be higher when the surrounding field is above or below the level of the test object than when it is equal. Cobb obtained just such results.

The right hand graph in figure 10 shows that when the brightness of J and K in figure 8b is increased the threshold for the bisecting border continues to fall and does not show a subsequent increase when the brightness of J and K exceeds the level of M and N . The explanation of this phenomenon seems to be that the extreme borders of J and K set up activities which spread across the squares and act on the ends of the bisecting border, lowering its threshold. But the possibility has not been eliminated that the effect is mediated by changes at the inner borders of the squares, although it seems that if this were the case a reversal would have occurred as the brightness level of the squares passed by that of the test object.

A good method for investigating this problem would be to leave the brightness of C constant in figure 9 and investigate the effect of varying the diameter of C . The nearer the outer edge of C to the test object, the lower should be the threshold. Our apparatus was not adapted to the problem, however.

SUMMARY

Experiments have been devised to study the effect of one border in the visual field upon the threshold of a neighboring border. When the activating border acts on the sides of the test border it tends to raise its threshold; when it acts on its ends it tends to lower its threshold. The activity which spreads from one border to another can be blocked by interposing a

third border between them. We are indebted to Drs. G. H. Bishop and P. W. Cobb for their criticisms and suggestions.

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REFLEX RESPONSES OF THE NICTITATING MEMBRANE

A. ROSENBLUETH AND H. G. SCHWARTZ¹

From the Laboratories of Physiology and the Department of Anatomy in the Harvard Medical School

Received for publication March 25, 1935

Reflex activity of the nictitating membrane (n.m.) of the cat may involve either the extrinsic or the intrinsic muscles. In the former case, protrusion of the n.m. is obtained on mechanical stimulation of the cornea. The muscles active in this reflex are skeletal, chiefly the external rectus (Rosenblueth and Bard, 1932). The intrinsic musculature of the n.m., however, is smooth. It is supplied exclusively by sympathetic nerves (Rosenblueth and Bard, *loc. cit.*), and is responsible for the usual state of tonic contraction of the n.m. It is possible under appropriate experimental conditions, to be described, to obtain reflex contraction (central excitation) and relaxation (central inhibition) of the intrinsic musculature. The present paper deals with such reflexes. Participation of skeletal muscle was excluded by injecting curare in control experiments.

METHOD. Cats were used. They were anesthetized with ether and the femoral vein was exposed. A cannula was inserted into the trachea. The ether was then discontinued and urethane (1.5 gm. per kgm.) was slowly injected intravenously.

In all experiments the adrenal glands were exposed through a midline abdominal incision and were tied off. In some cases the vagi were severed in the neck and the carotid-sinus nerves were cut at their origin in the arteries.

The movements of the right n.m. were recorded isotonicly by means of a light lever, after fixation of the head with a Czermak holder. The blood pressure (b.p.) was recorded from a carotid or a femoral artery by a mercury or a calibrated membrane manometer (Hürthle).

The nerves employed as afferents were cut peripherally, and buried shielded electrodes were applied to them. The stimuli were induction shocks at tetanizing frequency from a Harvard inductorium, or condenser discharges of variable capacities and intensities and at frequencies regulated by a metronome, or, finally, rectangular waves of variable intensities, durations and frequencies from a "multivibrator" circuit.

¹ Medical Fellow of the National Research Council.

RESULTS. A. *Reflex contraction.* The n.m. contracts readily after stimulation of all the afferent nerves thus far tried (Rosenblueth and Acheson, in preparation): cutaneous afferents (e.g., the saphenous), muscular afferents (e.g., the hamstring nerves), mixed somatic afferents (e.g., the sciatic) and visceral afferents (e.g., the splanchnics). In the present report we shall mainly describe observations in which the sciatic was used as an excitatory afferent. The general statements may be made that higher intensities and higher frequencies (up to 100 per second) of stimulation are more efficient in eliciting reflex contractions than lower intensities or frequencies and that the responses are a function of the intensity (spatial summation) or frequency (temporal summation), but these statements require qualifications which will be reported in later studies.

Single maximal shocks applied to the sciatic may evoke detectable reflex responses. As a rule, however, especially if smaller afferent nerves are employed—e.g., the saphenous—single afferent volleys are insufficient, and to evoke reflexes repetitive stimulation is necessary.

B. *Tonic contraction.* In the experimental conditions described the innervated n.m. is persistently semi-contracted as a consequence of a continuous tonic discharge of nerve impulses from the cervical sympathetic. If this nerve is severed the membrane relaxes. The degree of tonic contraction is usually moderate, about $\frac{1}{2}$ to $\frac{1}{4}$ of the maximal contraction of which the n.m. is capable. Not infrequently there are slow minor changes in the degree of tonic contraction. One of the factors responsible for these changes may be the depth of anesthesia; as a rule the tone was greater in lighter than in deeper anesthesia.

C. *Reflex relaxation.* Central inhibition of the tonic discharge described in the preceding section leads to a relaxation of the n.m. It is not easy to obtain from the afferents mentioned in section A; which lead to contraction; but it can be obtained in certain instances, of which figure 1A is an example. Low intensities and frequencies of stimulation are indispensable for inhibitory effects from these nerves.

Stimulation of the vagi (including the depressor fibers), on the other hand, readily leads to central inhibition and corresponding relaxation of the n.m. The effect may appear as a lessening of the tonic contraction (fig. 1B), as a depression of reflex contraction (fig. 2), or, finally, as a shortening of the after-discharge of a reflex contraction (fig. 3).

D. *The influence of the circulatory proprioceptors.* The ease with which afferent stimulation of the vagus elicits central inhibition (figs. 1, 2 and 3) suggested that its depressor component might be responsible. This idea further suggested that other circulatory proprioceptor afferents, e.g., the carotid-sinus nerves, might likewise inhibit the n.m. Afferent stimulation of the sciatic capable of eliciting reflex contraction of the n.m. is

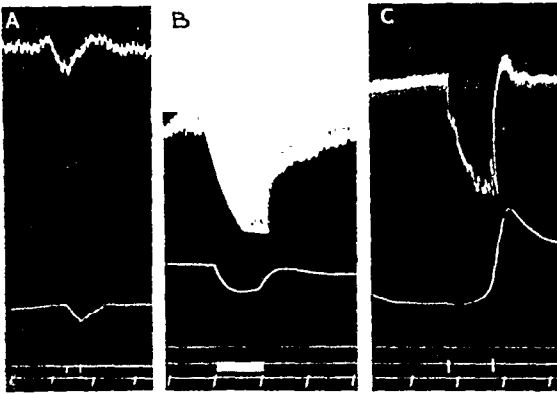


Fig. 1

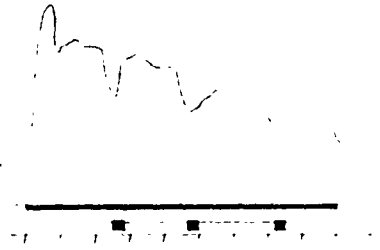


Fig. 2

Fig. 1. Reflex relaxation of the nictating membrane. In this and the succeeding figures the cats were anesthetized with urethane and the adrenals tied off. The time (lowest tracing) is recorded in 30-second intervals.

A. Vagi cut. Carotids denervated. At signal right hamstring nerves stimulated centrally. Multivibrator, 4 volts, 12 per second.

B. Vagi cut. Carotids denervated. At signal left vagus stimulated centrally. Inductorium, coil distance: 7 cm.

C. Same animal as in B. At signal thorax compressed by hand.

Fig. 2. Carotids denervated. Vagi cut. At upper signal right sciatic stimulated centrally with condenser discharges; voltage: 10; capacity: $0.1\mu\text{F}$; frequency: 2 per second. At the lower signals the left vagus was stimulated centrally with induction shocks, coil distance: 10 cm.

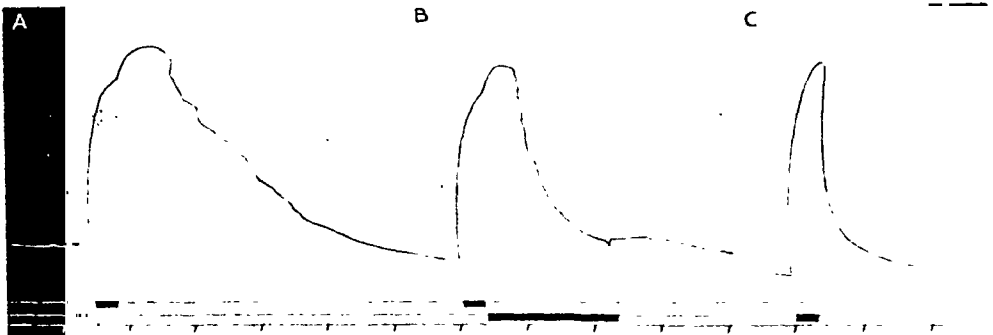


Fig. 3. Carotids denervated. Vagi cut.

A. Central stimulation of right sciatic. Inductorium, coil distance: 7 cm.

B. Same as A (upper signal) succeeded by central stimulation of left vagus, coil distance: 6 cm. (lower signal).

C. Peripheral stimulation of cut cervical sympathetic in the same animal. Coil distance: 11 cm.

usually attended by a rise of b.p. (figs. 4, 6 and 8D). If the suggestions just made were true, this rise of b.p. should set up depressor afferent impulses which would decrease the response of the n.m. Circulatory de-

afferentation should then lead to increased responses of the n.m. to a given stimulus. To test these ideas preliminary observations were made in animals in which the two vagi and the *right* carotid-sinus nerve were severed. Stimuli were applied to the sciatic and their effects were recorded with the *left* carotid artery open and closed. Figure 4 illustrates such an experiment and shows no significant differences between the responses obtained in the two conditions.

If the responses to sciatic stimulation are recorded, however, before and after section of both vagi and both carotid-sinus nerves, a striking increase of the reflex contractions obtains after the section (fig. 5).

E. Rebound. An excitatory rebound, i.e., a sudden increase of contraction at the end of stimulation, is frequently encountered (fig. 6). High

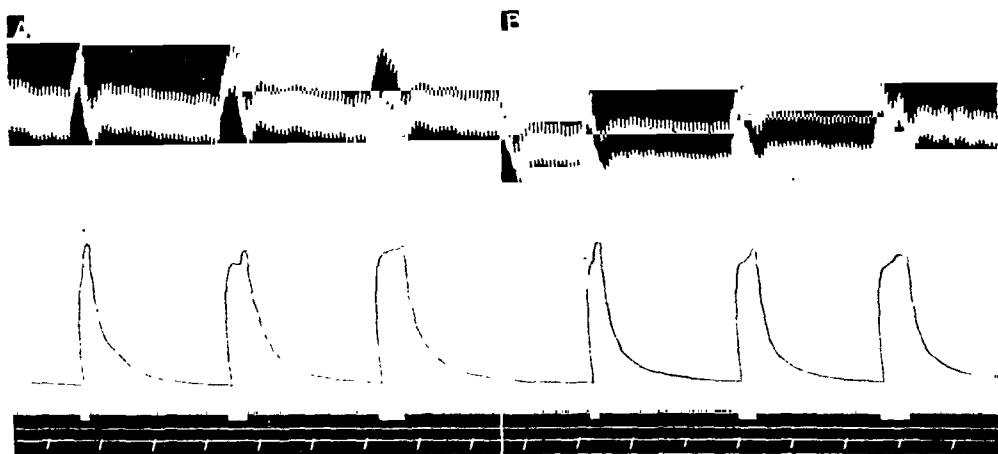


Fig. 4. Right carotid denervated. Vagi cut.

A. Central stimulation of the right sciatic (coil distance: 8 cm.) for 5, 10 and 15 seconds. The left common carotid was clamped throughout these observations.

B. The clamp on the left carotid was released. Same stimuli as in A.

intensities of stimulation are especially favorable for this excitatory rebound. An inhibitory rebound (i.e., a relaxation below the tonic basal level after the initial contraction, which occurs during stimulation) is, on the contrary, rare and not marked. Figure 8B illustrates an instance.

F. After-discharge. Relaxation after direct stimulation of the cervical sympathetic is a rapid process, lasting as a rule less than 1 minute (fig. 3C). The subsidence of a reflex contraction is usually longer, and may attain a duration of several minutes (fig. 3A). This long subsidence implies a long after-discharge from the centers. A prolonged after-discharge is also illustrated by the responses in which an excitatory rebound occurs (fig. 6).

G. Summation of reflex responses. If two excitatory afferents are stimu-

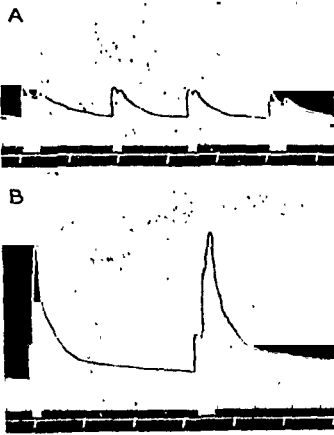


Fig. 5

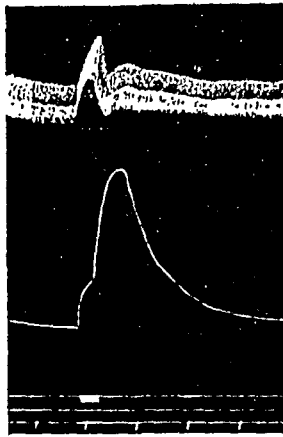


Fig. 6

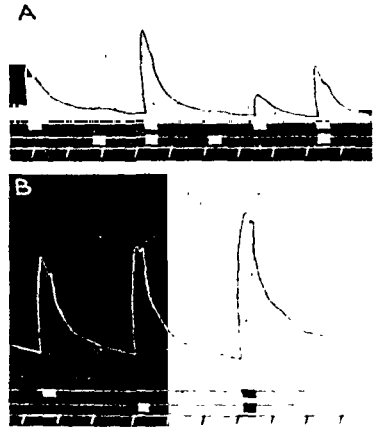


Fig. 7

Fig. 5. Responses to 5 and 10 seconds' stimulation of the right sciatic centrally (coil distance: 6 cm.).

A before and B after section of the vagi and depressors and carotid-sinus nerves.

Fig. 6. Right sciatic stimulated centrally (coil distance: 8 cm.).

Fig. 7. Carotids denervated. Vagi cut. The upper signal denotes central stimulation of the right sciatic and the lower of the left.

A. Coil distances: 13 cm.

B. Coil distances: 8 cm.

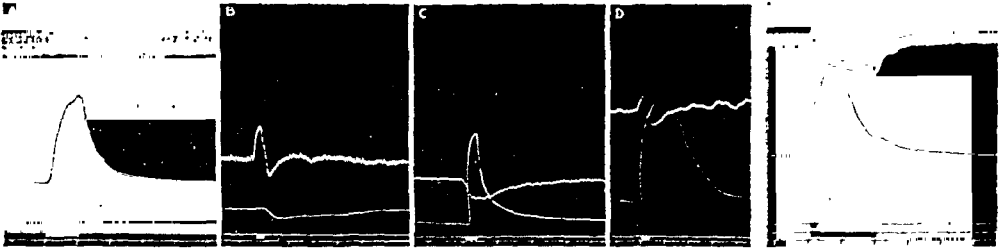


Fig. 8

Fig. 9

Fig. 8A. Central stimulation of the right sciatic. Multivibrator, 20 volts, 2 per second.

B. Central stimulation of the abdominal sympathetic chains at L5. Multivibrator, 4 volts, 20 per second.

C. Central stimulation of the hamstring nerves. Multivibrator, 26 volts, 10 per second.

D. Central stimulation of the right sciatic. Multivibrator, 8 volts, 20 per second.

Fig. 9. Carotids denervated. Vagi cut. Upper signal: right sciatic stimulated centrally (coil distance: 8 cm.). Lower signal: left vagus stimulated centrally (coil distance: 7 cm.).

lated first separately and then simultaneously, the response to the two stimulations is as a rule larger than that to either one alone. This summation is usually greater than lineal for minimal stimuli (facilitation) and

very scant for more intense and frequent stimuli (occlusion). Figure 7 illustrates typical instances.

H. *Lack of parallelism of the b.p. and n.m. responses.* All afferents thus far studied have effects on both the b.p. and the n.m. These effects are frequently parallel, i.e., contraction of the n.m. is associated with a rise of b.p. and relaxation with a fall (figs. 1A, 1B and 4).

The parallelism is, however, not invariably present. Figure 6 shows an instance in which it was absent. Furthermore, a definite change in either n.m. or b.p. can be obtained without any change in the other (fig. 8A and B), or they may respond in opposite directions (fig. 8C). The increased rises of b.p. in figure 4 after clamping of the left carotid, while the contractions of the n.m. were not modified, offer another instance of lack of parallelism. Still another interesting illustration of the independence of the two responses is seen in figure 8D, where an excitatory rebound in the n.m. was associated with an inhibitory rebound in the b.p.

This independence of the reflex effects was further tested by lowering of the b.p. while a reflex contraction of the n.m. was induced. Figure 9 records excitatory effects on the n.m. and a lowered b.p. produced by simultaneous stimulation of the sciatic and the vagus. Figure 1C shows that lowering the blood pressure by compressing the chest and opposing the venous return to the heart does not induce a relaxation of the n.m., but a contraction, probably due to asphyxia.

DISCUSSION. All the features of the reflex responses of the n.m. described in the preceding sections are features likewise found in spinal reflexes (cf. Sherrington, 1906). The differences which may be met between autonomic and somatic spinal reflexes are quantitative, not qualitative, thus suggesting that the fundamental mechanisms underlying the reflex responses are probably identical for the two classes. The most striking quantitative difference discernible is in the time parameters, especially marked if we compare the intervals for effective central summation and the after-discharges. The source of this difference is yet unexplained. The fact remains that by multiplying the respective time scales by suitable factors all reflex responses thus far studied conform to a common pattern and this analogy of features is in favor of an analogy of processes.

One of us (Rosenblueth, 1934), impressed by the prolonged after-discharges occurring in cardiac reflexes, suggested that they are more readily explained, and with less subassumptions, if we postulate that the central excitatory state (c.e.s.) may attain supraliminal values (as could be the case if the central excitatory agent were a chemical substance—cf. Sherrington, 1925), than if we postulate only a liminal c.e.s. and a persistent bombardment of the neurones. In the present report we are again confronted by after-discharges which may last several minutes (figs. 3A, 6 and 8D). They are a direct consequence of the nerve impulses afferent

to the centers and not an indirect effect of other changes induced by the stimuli, such as alterations of blood pressure (section H, figs. 8 and 9). Our opinion is again that, although after-discharge might be due to a continuous bombardment by impulses traveling over self-reëxciting, reverberating paths (Ranson and Hinsey, 1930), it can be more simply explained in terms of a chemical mediation at the synapses. A firmer judgment must await direct evidence for either, or both, or alternative theories.

Rebound was obtained from all the excitatory afferents used (section A). This leads to the inference that all these nerves are mixed, containing both excitatory and inhibitory afferents (see Sherrington and Sowton, 1911). The mixed nature of the nerves makes uncertain the interpretation of the phenomena of summation (section G). For example, the rôle which inhibition might play in a response showing apparent occlusion (fig. 7B) can not be determined without further experimental analysis.

Information is inadequate with regard to the receptors from which the stimulated afferent nerves arise. Accordingly, little may be said concerning the physiological significance in the organism of the reflexes which we have studied.

An interesting property common to these and other autonomic reflexes is the frequent lack of "local sign." Some vasomotor reflexes bear a local sign, e.g., the Lovén and the axon reflexes; many others, however, both pressor and depressor, do not. The effects are then generalized, and no specific changes are noticeable in the territory where the afferent originates. Cardiac reflexes do not bear a local sign, nor do the n.m. reflexes here described.

Reflex contraction of the n.m. coincides as a rule with acceleration of the heart, rise of b.p., secretion of adrenine, dilatation of the pupil, salivation, etc. In all these responses an increased sympathetic discharge is or may be involved. On the other hand, erection of the hairs has not been noted; and it is possible to separate some of the responses (section H). It appears, therefore, that the effects are not obtained from stimulation of a main sympathetic center but from independent centers concerned with the different functions mentioned. A more detailed study is necessary, which should include a comparison of various anesthetics; thus cardiac and vasomotor reflexes are readily obtained under chloralose, but reflex contraction of the n.m. is absent. The principle of generalized sympathetic activity (Cannon, 1930) is, however, at present the only way of accounting for contraction of the n.m. on stimulation of a saphenous or a hypogastric nerve.

SUMMARY

Reflex contractions and relaxations of the smooth muscle in the nictitating membrane of the cat are described. The vagus is a suitable afferent to

demonstrate reflex relaxation (figs. 1, 2 and 3). Section of the vagi and denervation of the carotids increase the contractions elicited from the sciatic (fig. 5). Excitatory and inhibitory rebounds may be recorded (figs. 6 and 8B). After-discharge may be very prolonged (figs. 3A, 6 and 8D). Simultaneous stimulation of two afferents leads to summation of the reflex effects (fig. 7). The responses of the nictitating membrane are direct and not secondary to changes of blood pressure (figs. 1, 4, 6 and 8).

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THE EXTRAHEPATIC BILIARY TRACT DURING ANAPHYLAXIS

KARL DEISSLER AND GEORGE M. HIGGINS

From the Division of Experimental Medicine, The Mayo Clinic, Rochester, Minnesota

Received for publication March 22, 1935

This report covers a study of the gall bladder during anaphylaxis following protein sensitization. It comprises three parts: 1, the reaction of the empty gall bladder to the antigen when the vesicle was suspended as a strip in a physiologic solution; 2, the pressure induced within the gall bladder when antigen was added to the bath in which the vesicle was suspended; and 3, the reaction of the sphincteric mechanism, at the duodenal end of the common bile duct, to the antigen when it was added to the bath.

Ivy (1) has recently made a most excellent review of the literature on the physiology of the gall bladder, and there appears to be but little experimental work directly bearing on this phase of physiology of the gall bladder during anaphylactic shock.

METHODS. Young male guinea pigs that had been previously sensitized by the subcutaneous injection of 0.1 cc. of horse serum and 0.1 cc. of egg white (chicken) were used. The guinea pigs were ready for use about three weeks after the injection.

The animals were killed by a blow on the head, the abdomen was quickly opened, and the extrahepatic biliary tract, together with the portion of duodenum containing the sphincter of the common bile duct, was removed and placed in Tyrode's solution. At the same time, a small segment of intestine and of the urinary bladder were removed, and in some experiments these were tested for the anaphylactic reaction, along with the gall bladder. In part 1 the gall bladder was severed from the rest of the biliary tract, all bile was expressed, and then one end was tied to an arm of a small glass frame. The strip of intestine and the strip of urinary bladder were likewise tied to adjacent arms of this glass frame, and then all were suspended in a bath of 50 cc. of Tyrode's solution, which was maintained at a constant temperature of 38.5°C. The other ends of the three structures were each tied to a lever in a manner usually employed for recording the movements of intestinal strips. In part 2 the gall bladder was cannulated, perfused a number of times to free it of all bile, and then was immersed in the bath. By means of a small rubber tubing, one arm of a

T-tube connected the incannulated gall bladder with a buret, while the other arm led to a water manometer that was fixed along the front elevation of the photographic kymograph that was used to record the changes which were observed. The buret served as a convenient means of filling, or removing fluid from, the gall bladder. The strip of intestine was fixed to the glass frame, as in part 1, immersed in the bath along with the gall bladder, and tied to a recording lever, the end of which moved freely in front of the manometer. Thus, the movements of the water column in the manometer and the movements of the lever were recorded photographically at the same time. In part 3 the extrahepatic biliary tract was removed in continuity with that portion of the duodenum that contained the sphincter of the common duct. The hepatic duct, just above its junction with the cystic duct was incannulated and all other biliary tributaries were ligated. After washing the biliary tract free of all bile, this preparation was immersed in a bath of 50 cc. of Tyrode's solution and connected by means of the T-tube to the buret and to the manometer as described previously. A strip of intestine was likewise attached to the recording lever. The biliary tract preparation was so arranged, when suspended in the bath, that the neck of the gall bladder and the duodenal segment, including the sphincter, were at the same level. We employed our photographic recording device (2) to record all changes that were induced in these organs during the reaction to the antigens.

In order to test for the anaphylactic response in these preparations, the antigen, either horse serum or egg white, was added directly to the 50 cc. physiologic bath in which the tissues were suspended. The amount of the antigen which was added was always first diluted by adding enough of the bath solution to make 1 cc., and then was added to the remaining 49 cc., so that the concentration of the specified antigen was the actual concentration in 50 cc. of the solution.

EXPERIMENTAL OBSERVATIONS. 1. *The reaction of the empty sensitized gall bladder to the antigen.* In order to secure adequate oxygenation and a normal muscle tonus, the gall bladder, urinary bladder, and intestinal strip were allowed to remain in the Tyrode's solution for fully fifteen minutes before testing for the anaphylactic response. When horse serum, in a dilution of 1 to 2500, was added to the bath solution in which these organs were immersed, there was a marked contraction (fig. 1, *no. 1*). The response of the intestine was more immediate than that of the urinary bladder or of the gall bladder, and in all three there was a brief latent period before the reaction to the antigen was manifested.

After changing the solution twice and after a brief rest period, during which the organs regained their normal tone, egg white was added to the bath (fig. 1, *no. 2*). A concentration of 1 to 5000 of egg white produced a comparable, but more marked, contraction of all three organs.

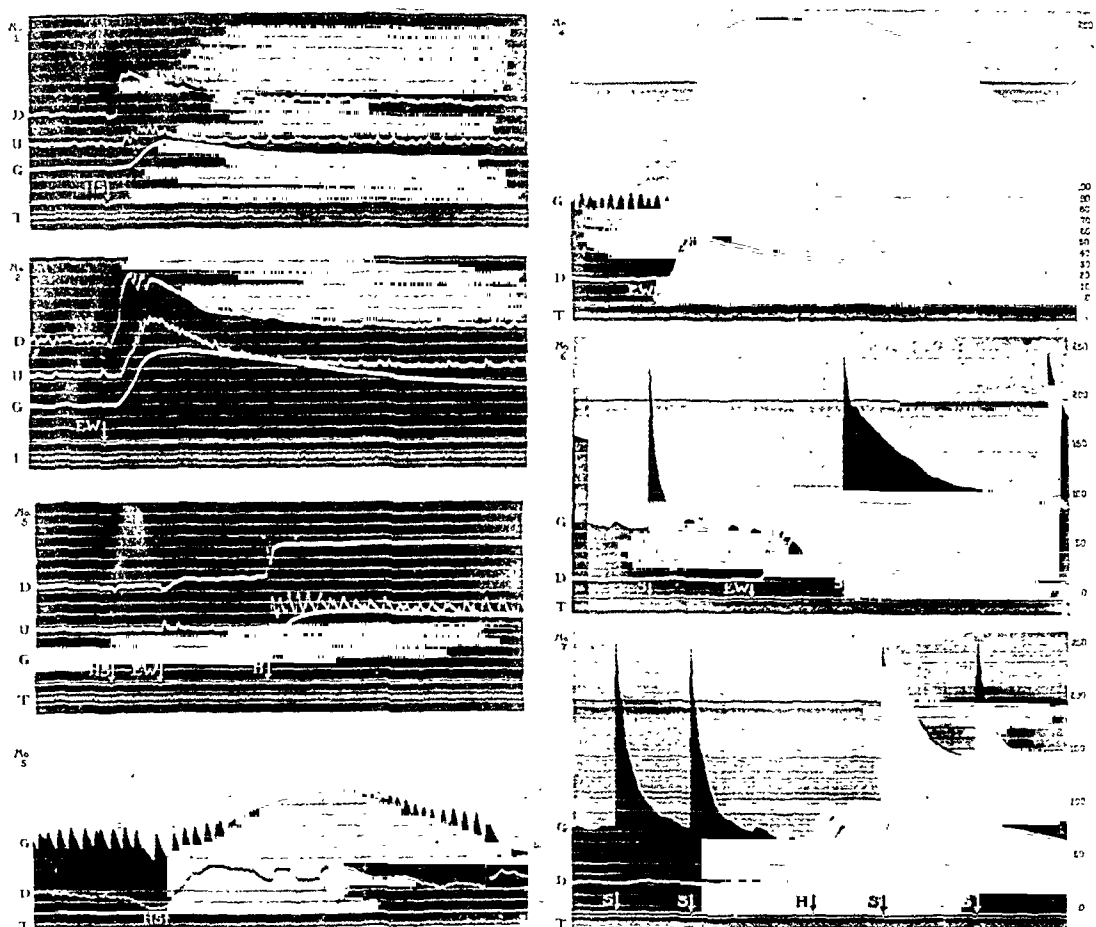


Fig. 1. In all tracings *D* represents duodenum; *U*, urinary bladder; *G*, gall bladder; *T*, time (between long dashes equals 1 minute).

Nos. 1 and 2. Same preparation in both. Effect of adding different substances to the bath: *HS*, horse serum, 1 to 2500; *EW*, egg white, 1 to 5000.

No. 3. Same preparation as that in nos. 1 and 2. It is evident that the preparation had been desensitized to horse serum and to egg white by the procedures represented in nos. 1 and 2. Addition to the bath of histamine, *H*, 1 to 100,000, demonstrated viability of the preparation.

Nos. 4 and 5. Pressure within the gall bladder, measured in millimeters of water. In no. 4, *EW* represents egg white, as before. The animal represented in no. 5 had been weakly sensitized to horse serum. *HS*, horse serum added to bath.

Nos. 6 and 7. Reaction of sphincter of common bile duct. Records are in millimeters of water. No. 6, *s* represents test of resistance offered by sphincter before inducing anaphylaxis by adding the antigen, egg white, *EW*, to the bath; *s'*, after anaphylaxis. No. 7. Reaction of sphincter before, *s*, during, and after *s'*, addition of histamine, *H*, 1 to 100,000 to the bath.

In order to determine whether this was a true anaphylactic response, we changed the solution in the bath three times, adding fresh Tyrode's solution each time, and then added a concentration of the horse serum three times as great as that which was added before. This time there was no contraction of either the gall bladder or the urinary bladder and only a slight response by the intestinal strip (fig. 1, *no. 3*). Likewise, when a concentration of egg white that was three times as great as that which was used the first time was added to the bath, the gall bladder did not contract, although there was a slight response by the urinary bladder and by the intestinal strip. Thus, desensitization had occurred and the responses, which are recorded in figure 1, *nos. 1 and 2*, and which followed the addition of the antigens, were typical anaphylactic responses. In order to test the viability of these organs, we added histamine to the bath, in a concentration of 1 to 100,000 (fig. 1, *no. 3*). There was an immediate response and all organs contracted without any intervening latent period. This immediate reaction was typical of all histamine reactions and different from the anaphylactic changes which follow after a brief latent period.

2. *Intra-gall bladder pressure during anaphylaxis.* Although our first experiments had demonstrated clearly an anaphylactic response by the gall bladder to either horse serum or egg white, we wished to observe the changes in pressure that occurred within the vesicle during these responses, and to compare them with the pressure which occurs in a normally contracting vesicle.

By means of the buret and the T-tube connection with the gall bladder and the manometer, as described previously, the biliary vesicle was slowly filled with the physiologic solution until such pressures, at which normal gall bladder rhythm was induced, were reached. On the addition of 0.01 cc. of egg white to the 50 cc. Tyrode bath, making a concentration of 1 to 5000, there was a marked contraction of the gall bladder and the intestinal strip. The gall bladder pressure rose rapidly from 75 mm. to 250 mm. of water. This high pressure level was maintained for three minutes, when a gradual relaxation occurred and the pressure was gradually reduced (fig. 1, *no. 4*). We encountered this rather powerful contraction of the gall bladder, which raised the pressure to 250 mm. of water, only once, but usually during such an anaphylactic response the pressures increased from 60 or 70 mm. to 160 or 200 mm. of water. These pressures, during anaphylaxis, were invariably higher than those which a contracting gall bladder of a guinea pig usually exerts and they probably represent the maximal pressure against which the vesicle may contract.

After the preparation had been washed, we tested for the anaphylactic response a second time, using a triple concentration of the same antigen. As there was no response or contraction by the gall bladder, it was certain that desensitization had occurred, and that the contraction we had ob-

served was definitely the result of anaphylaxis. When we added histamine to the bath solution, in a concentration of 1 to 100,000, the gall bladder contracted immediately, thus demonstrating the viability of the preparation.

When animals were weakly sensitized to either protein, or when submaximal doses of antigen were added to the bath containing the isolated gall bladder, only slight contractions of the viscus were observed. In the experiment (fig. 1, *no. 5*), the animal had been weakly sensitized to horse serum. On the addition of the antigen, in dilute concentration to the bath in which the gall bladder was suspended, there was an immediate and sustained reaction by the duodenum but a more gradual response by the gall bladder. This retarded reaction of the gall bladder showed clearly the relation of rhythm to tonic contraction. During the two-minute interval after the addition of the antigen to the bath, there was a slight increase in the tonic contraction of the vesicle as evidenced by an increase in pressure without any appreciable change in either the frequency or the amplitude of rhythm (fig. 1, *no. 5*). As tonic contraction increased and as the intravesical pressure rose from 40 to 100 mm. of water, the amplitude of rhythm gradually decreased. The frequency of rhythm, however, increased from 3 or 4 cycles per minute to about 8 or 10 per minute. After the full force of the contraction had passed and a gradual relaxation was in progress, even without washing, a rhythm of normal amplitude and frequency returned (fig. 1, *no. 5*). Rhythm was dependent on an optimal pressure within the gall bladder and will not occur at intravesical pressures either too low or too high.

3. *The reaction of the sphincter of the common bile duct during anaphylaxis.* When animals were observed during an anaphylactic response, we found that the gall bladder was usually well distended with bile. Since the vesicle, *in vitro*, did contract, often with exceedingly high pressures, in the presence of antigen, it was of interest to know whether the sphincteric mechanism at the duodenal end of the common bile duct likewise contracted and perhaps inhibited the evacuation of the vesicle in the intact animal. We wished to know whether or not the gall bladder pressure during an anaphylactic response was greater or less than that which is exerted by the regulatory sphincter at the duodenal end of the common bile duct.

The extrahepatic biliary tract, together with a segment of duodenum which contained the common duct sphincter, was suspended in the Tyrode bath in the manner described previously. After a brief rest interval to allow for thorough oxygenation, the gall bladder was slowly filled through the cannula attached to the buret. The pressure in the preparation was slowly increased and, when pressures of 70 or 80 mm. of water were recorded on the manometer, rhythmic contractions were evident in the gall bladder. The sphincteric mechanism withstood these pressures and fluid

did not pass through the ampulla into the bath; but if more fluid was added from the buret, so as to increase greatly the pressure, all rhythm in the gall bladder then ceased and the resistance of the sphincteric mechanism was overcome, thus permitting the escape of the fluid.

The resistance of the sphincteric mechanisms was tested in the following manner: the physiologic solution was permitted to flow from the buret into the gall bladder preparation until a pressure of 230 mm. of water was recorded on the manometer. With such a pressure the gall bladder was overdistended and the resistance of the sphincter was overcome, so that fluid immediately passed from the preparation through the ampulla and the pressure immediately decreased (see first part of fig. 1, *no. 6*). When pressure levels, which the sphincter normally withstood, were reached, the ampulla closed, no further fluid escaped into the bath, and rhythmic contractions were resumed by the gall bladder (fig. 1, *no. 6*). The pressure exerted by the sphincteric mechanism was quite constant and hovered around the level at which normal rhythm occurred.

Having learned what the normal resistance offered by the sphincter for any such preparation was, we were prepared to test the reaction of the sphincteric mechanism during anaphylaxis. When egg white, in a concentration of 1 to 1000, was added to the bath, there was a brief latent period, which was followed by a typical response in both duodenal and hepatic duct preparations (fig. 1, *no. 6*). The immediate increase in pressure from 60 to 93 mm. represents the maximal contraction of the gall bladder during its response to the antigen. After about two minutes, the pressure in the system was raised, by adding fluid from the buret in the manner previously indicated, to about 250 mm. The effect, which was induced on the sphincter by the antigen, was clearly demonstrated by the contour of the curve which was described by the water level as it much more slowly descended in the manometer (fig. 1, *no. 6*). Whereas it required but one minute, in the test prior to adding antigen, for the fluid in the manometric system to pass the ampulla and to come to rest at the usual pressure level, it required about five minutes after the antigen had induced a contraction of the sphincter. But what is more significant is that the pressure, which was withstood by the sphincteric mechanism during the anaphylactic response, was 40 mm. more than before, and was greater by 10 mm. than the maximal pressure which was exerted by the gall bladder. This result occurred in all experiments undertaken and showed definitely that during an anaphylactic response the resistance which was offered by the sphincter at the duodenal end of the common bile duct was greater than the emptying power of the gall bladder. Thus it may be that during anaphylaxis the gall bladder does contract but may not empty because of the inhibiting power that is exerted by the sphincter at the end of the common bile duct.

When we added histamine, in a concentration of 1 to 100,000, to a bath which contained such a preparation of the extrahepatic biliary tract that had been washed repeatedly, a similar result was attained (fig. 1, *no 7*). In this case, there was no latent period; the strip of duodenum responded in the usual way to histamine and the pressure within the gall bladder rose from 65 to 100 mm. of water. When we tested the sphincteric resistance by again filling the manometric system to 250 mm. and permitting fluid to flow through the ampulla, we learned that the resistance during histamine response had increased about 70 mm. and that the sphincteric mechanism now withstood a pressure of 140 mm. of water. The pressure which was exerted by the sphincter during histamine response exceeded that which was exerted by the gall bladder by about 40 mm. of water. Thus, in the histamine reaction, as in anaphylaxis, the resistance that is offered by the sphincter at the duodenal end of the common bile duct is greater than the force that is exerted by the contraction of the gall bladder, and probably is of significance in explaining why the gall bladder fails to empty.

SUMMARY

This report covers a series of observations on the isolated extrahepatic biliary tract of guinea pigs, during anaphylactic responses to foreign proteins to which the animals had been previously sensitized.

All animals were sensitized by the subcutaneous injection of 0.1 cc. of horse serum and 0.1 cc. of (chicken) egg white. Three weeks later, they were killed and the extrahepatic biliary tract was removed, placed in Tyrode's solution, and subsequently tested for the anaphylactic response.

The observations reported in this paper warrant the conclusions that the biliary tract, including the gall bladder, may become sensitized to foreign proteins equally as well as any other organ, and that it will contract vigorously in the presence of the antigen.

When antigen in a concentration of 1 to 2500, or 1 to 5000, was added to the bath of Tyrode's solution in which the gall bladder was suspended, contraction of the vesicle followed after a brief latent period. This was true of a strip of duodenum and a strip of the urinary bladder, which likewise were suspended in the bath with the gall bladder.

When the antigen was added to the bath in which a rhythmically contracting gall bladder was suspended, the intravesical pressure immediately rose from levels of 60 or 70 mm. of water to levels as high as 200 or 240 mm. of water. These are pressures which are higher than those usually encountered in a normally contracting gall bladder.

When antigen was added to the bath in which the entire extrahepatic biliary tract, together with the sphincter at the duodenal end of the common bile duct, was suspended, there was a contraction by both the gall bladder and the duodenal sphincter. The force or pressure which was

exerted by the sphincteric mechanism was found to be greater than that which was exerted by the gall bladder. This may explain the observations hitherto made, that the gall bladder ordinarily does not empty during an anaphylactic shock. The gall bladder does contract, but the inhibitory influence of the sphincteric mechanism prevents the flow of bile into the duodenum.

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THE EFFECT OF INTRAVENOUS INJECTIONS OF AMINO ACIDS ON THE MOTILITY OF THE STOMACH IN NORMAL AND FASTING DOGS

HARRY BOWMAN, J. F. REGAN AND EUGENE U. STILL

From the Department of Physiology, University of Chicago, Chicago, Illinois

Received for publication April 1, 1935

There have been many theories expressed concerning the cause of hunger contractions of the empty stomach. The relationship of the level of blood sugar to the hunger activity has been investigated by many workers. There is not a unanimity of opinions among them. A bibliography will be found in the papers of Mulinos (1933), and LaBarre (1931). It is not clear to us just what is the cause, or causes, of the periodic gastric motor activity seen in hunger.

Being unaware of any studies on the effect of amino acids upon the hunger contractions, it seemed possible that data along that line might to some degree throw light upon the question.

METHOD OF STUDY. Three healthy dogs, having gastric fistulae, and weighing between 12 and 14 kgm., were used. They were thoroughly trained to lie quietly on a table while motility tracings were being made by the balloon method. The same general procedure and equipment were used in these experiments as have been used in various papers reported from this laboratory in recent years.

The animals were allowed to go without food for a period of eighteen to twenty-four hours before each experiment. Normal controls were run on each animal for several days before injections of amino acids were made. Injections of saline were used to rule out the effect of the injection per se.

Various amino acids were prepared by one of us (E. U. S.), and recrystallized until the constants closely approximate the values accepted for the pure substances. The amino acids were dissolved in a small volume of neutral saline. Injections were made at various times with respect to the state of gastric motility, i.e., at the beginning of a quiescent period, at the beginning of activity, at the height of activity or at the end of activity. Table 1 shows the results of this study. This table was constructed so as to show how long the particular activity of the stomach had been going on at the time the injection was made; and how long after the injection before there was noticeable change.

The possibility was suggested that not a single amino acid was involved

TABLE 1

The effect of intravenous injection of amino acids on the motility of the empty stomach

AMINO ACID	AMOUNT	DOG NO.	LENGTH OF PERIOD PRECEDING INJECTION	MINUTES AFTER INJECTION BEFORE CHANGE IN MOTILITY
	<i>mgm.</i>		<i>minutes</i>	<i>minutes</i>
Glycine.....	100	1	57	125
	100	2	18	35
Cystine.....	21	1	30	60
	20	1	8	8
	30	1	9	11
	21	2	18	33
	20	2	32	110
	30	2	0	8
Leucine.....	50	1	5	13
	50	2	20	31
Isoleucine.....	50	1	30	40
	50	2	18	52
Tyrosine.....	25	1	15	51
	35	1	4	7
	25	2	56	53
	35	2	?	?
Proline.....	50	1	120	10
	50	2	131	40
Tryptophane.....	26	1	49	81
	26	2	10	45
Lysine.....	50	1	16	53
	50	2	15	56
Arginine.....	15	1	11	4
	35	1	17	12
	15	2	33	13
	35	2	6	83
Histidine.....	15	1	85	50
	21	1	90	21
	18	1	111	18
	15	2	92	43
	21	2	15	12
	18	2	24	29

TABLE 1—*Concluded*

AMINO ACID	AMOUNT	DOG NO.	LENGTH OF PERIOD PRECEDING INJECTION	MINUTES AFTER INJECTION BEFORE CHANGE IN MOTILITY
	<i>mgm.</i>		<i>minutes</i>	<i>minutes</i>
Glutathione.....	11	1		60
	15	1	34	35
	10	1		
	8	2	18	12
	15	2	32	30
	10	2	32	57
Creatine.....	50	1	18	17
	50	2		

but rather a group of amino acids. Therefore, we planned experiments based upon the assumption that hunger was abolished upon the absorption of the amino acids contained in a protein digest. We prepared a mixture of amino acids in the proportion found in casein. Solutions containing 600 mgm. of the mixture were injected intravenously under the conditions described above. There was no apparent effect upon the hunger motility of the stomach.

DISCUSSION. So far as we know, there are little data available on the concentration of various amino acids in the blood. Abderhalden (in 1913) showed, however, that proline, leucine, valine, alanine, glycine, aspartic acid, glutamic acid, tryptophane, lysine, arginine and histidine were present. The average figure for the non-protein amino nitrogen in the blood of the fasting dog is 4.4 mgm. per 100 cc. of blood. This indicates that about 31 mgm. of amino acids are found in 100 cc. of the blood. Assuming our dogs have a blood volume of 1 liter, the total circulating amino acids amount to 300 mgm. (fasting). Since at the height of absorption the amino acid content of the blood is about 100 per cent above the fasting level, the increase in our animals is estimated to be about 300 mgm.

In one group of experiments we have injected the individual amino acids in such amounts as would increase their particular concentration by 100 per cent or more. In another group of experiments we have injected a mixture of amino acids (approximating the composition of casein), in such amounts as would increase the total amino acid concentration of the blood by 200 to 300 per cent. Neither group of experiments indicated that the injections altered the state of gastric hunger motility, neither in the direction of augmentation nor inhibition. This was true when the injections were made during any phase or state of activity and, therefore, the state of the stomach was not considered to be a complicating factor.

CONCLUSIONS

Inasmuch as our experiments (43 in number) on three dogs have not provided evidence indicating that the length, amplitude or frequency of periods of gastric motility are modified by the intravenous injection of purified amino acids, we are of the opinion that the level of amino acids in the blood is not a contributing factor to the hunger contraction mechanism.

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THE DISAPPEARANCE OF DIGESTIVE INHIBITION WITH THE REPETITION OF EXERCISE¹

FRANCES A. HELLEBRANDT, ELIZABETH BROGDON²
AND SARA L. HOOPES

From the Department of Physiology, University of Wisconsin

Received for publication March 11, 1935

In our first experience with the effects of exercise upon the acidity of the gastric juice we noted that the depressing influence of muscular work progressively lessened with repetition of the effort (Hellebrandt and Miles, 1932). This was assumed to be a training effect. Subsequently the same observation was made under circumstances which definitely precluded calling it training in the usual sense of that term, which implies the development of adaptations facilitating the performance of work as a result of systematic, long repeated exercise. Inhibition vanished after a few trials of the prescribed work had been superimposed at long and irregular intervals upon the strenuous activity program of one inured to much exercise (Hellebrandt and Hoopes, 1934). Steinhaus (1931) had stressed the importance of emotional factors. There was reason to think that exercise without feeling does not have the same effect on gastric function as that associated with psychic stress. The object of this study was therefore, to emancipate exercise from its psychic concomitants in man; to observe the effects of work alone upon gastric secretory function; and finally to introduce the emotional element, either by itself or in conjunction with exercise.

METHODS AND RESULTS. The subjects were healthy young adult women, students or graduates of a professional course in physical education, habituated to vigorous exercise, familiar with the technique of gastric intubation and physiologic experimentation. There were 6 subjects upon whom 75 fractional analyses were made under resting conditions. Secretion was stimulated by 50 cc. of 7 per cent alcohol or by histamine. The methods of aspiration and titration were the same as in our previous work. The alcohol was warmed and introduced through the stomach tube. Samples of the gastric contents were removed at quarter hour intervals. Histamine was given hypodermically in a dosage of 0.01 mgm./kgm., using

¹ This work was made possible in part by a grant from the Wisconsin Alumni Research Foundation.

² From the Department of Physical Education for Women, University of Minnesota.

a 1:1000 solution of ergamine acid phosphate. The total secretion was aspirated every 15 minutes. Laboratory exercise was performed on 74 days, on 34 of which the gastric response was also studied. There were in addition 34 single aspirations, 6 under resting conditions and 28 associated with exercise. Observations were likewise made when natural situations accompanied by emotional disturbances were experienced. A few attempts were made to simulate psychically agitating occasions.

1. *Fractional analysis after alcohol.* It is difficult to find a measurable generalized exercise so monotonous and readily stereotyped as pedalling the stationary bicycle at a constant speed against a fixed load. Working for 15 minutes at 740 kgm. m./min. with an average pedalling rate of 73 R.P.M. approached the limits of endurance of our subjects. This work was performed on consecutive days by two subjects. Graph I, figure 1, illustrates the response of S. L. H. and shows by the sixteenth day the depression had disappeared. *Very severe exercise can be performed without inhibiting the acid secreting mechanism of the stomach.* Subject S. L. H. continued exercising daily, either alone or in the presence of technical assistants. On the 39th day she performed in public demonstration before a mixed audience. The experience was without effect upon the acidity response as shown in graph II, figure 1. *An emotionally upsetting situation may be met without suppressing gastric function.*

The repetition of work is associated with the acquisition of a trained state. Seasoned to meet the demands of a given physical task, the subject is only partially trained for more severe grades of effort (Steinhaus, 1933). If the eventual ability to carry on gastric work undisturbed during and after exercise is a part of the complex training phenomenon, increasing the difficulty of the muscular exertion should be accompanied by a reversion to the state of secretory inhibition. On the 44th consecutive day of exercise the subject preceded the usual 15 minutes of severe work by an exhaustive bout of exercise lasting for one hour. Graph III, figure 1, shows that this had almost no effect on the way in which her stomach secreted HCl in response to the standard test meal of alcohol. On the 46th day the severity of the routine effort was pushed to the limit of the subject's physiological ability, 1031 kgm. m./min. at a pedalling rate of 88 R.P.M. Circulatory and respiratory distress was acute. At the cessation of work there was perioral blanching, very labored breathing and threatened collapse. Graph IV, figure 1, shows that in spite of exercise violent to the point of prostration gastric function proceeded unimpaired. This is striking evidence that the mere contraction of muscle and the vasomotor shifts associated with generalized exercise are in themselves powerless to suppress this phase of gastric function.

Subsequently S. L. H. re-trained for the same exercise to note if freedom from suppression would come on more promptly. By the ninth day all

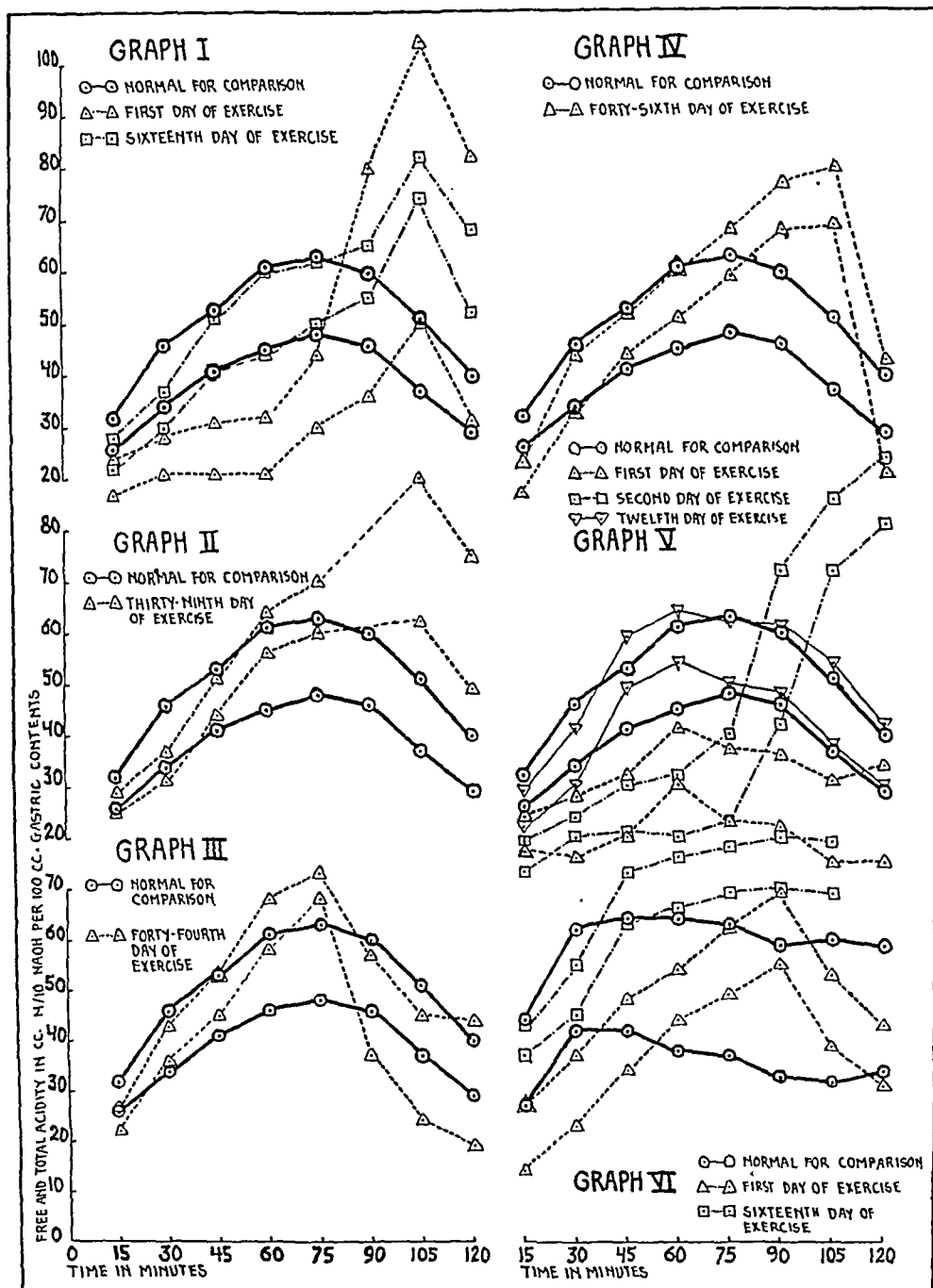


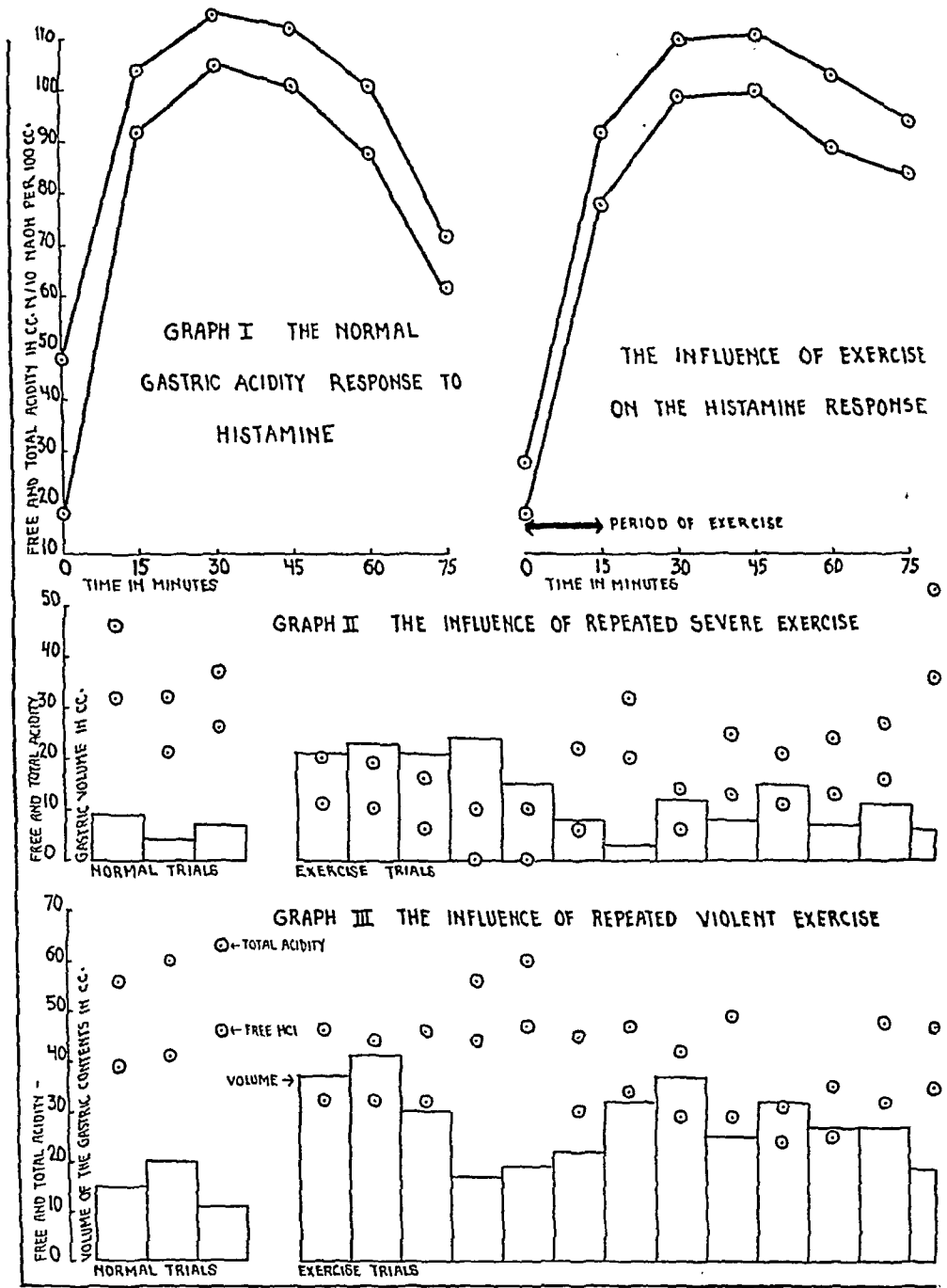
Fig. 1. Graphs showing the acidity response to a test meal of alcohol under the following conditions: I. Repetition of work by subject S.L.H. II. Psychic disturbance superimposed upon repeated work. III. and IV. Increasing the severity of the work. V. Confirmatory series of S.L.H. on repeated work. VI. Repetition of work by subject F.A.H.

evidence of the characteristic first hour inhibition had disappeared and by the twelfth the titration curve was approximately the same as that at rest (graph V, fig. 1). Graph VI, figure 1, demonstrates the main results of a similar series of observations on another subject. The exercise could not be carried out by F. A. H. without cardiac strain. Examination on the 16th day indicated enlargement to percussion and orthodiagraphic study confirmed the impression. It seems significant that work exhaustive enough to bring on cardiac enlargement should be associated with so little change in gastric function.

2. *Fractional analysis after histamine.* The administration of histamine was always accompanied by brilliant flushing, a sensation of heat, and transient throbbing headache. Unpleasant symptoms were notably alleviated by exercise. The second time work was performed immediately after the injection of histamine, the first post-exercise gastric sample was slightly discolored. The histamine juice at rest had been clear, colorless and limpid. As the experiment was repeated coloration increased until on the fourth trial all post-exercise samples were tinged to the hue of highly colored urine. Many contained a chocolate brown flocculent precipitate. All samples were positive for blood.

Histamine produces capillary dilatation (Krogh, 1930). That the permeability of the vessel wall is decreased is evidenced by the edematous induration at the site of the injection. The high systemic blood pressure of severe exercise may, under such conditions of capillary paralysis, be responsible for the appearance of blood in the stomach secretions. Bleeding was not due to trauma by the tube. We have examined more than 1200 gastric samples for occult blood and have ample evidence that in our tube-trained subjects the aspiration of the fasting residuum and the gastric contents after stimulation by gruel, alcohol or histamine may be accomplished without occult or visible bleeding. This is true also of post-exercise gastric aspiration when alcohol is the secretory stimulant. It may be of practical importance to know whether histamine is contraindicated in the presence of vascular hypertension or whether repetitious administration is harmful. Fluoroscopic and radiographic examination showed no gastric abnormality in the two subjects upon whom the observation was first made. At no time in the limited series of experiments completed did exercise significantly suppress the secretory response to this powerful stimulant. The exercise curves were like those obtained at rest as illustrated in graph I, figure 2.

3. *Single aspirations.* A one hour test breakfast of 300 cc. of warm water and 4 arrowroot cookies was administered in the usual way. Subject J. H. performed the severe 15 minute exercise immediately after its ingestion. At first distinctly depressing, the one hour acidity gradually returned toward and eventually superseded normal (graph II, fig. 2). The



large volumes of the 60 minute aspirations on the first few days of exercise indicate motor inhibition, which in itself contributes by dilution to the low acidity values. R. E. B. studied the effect of a short bout of violent work at high speed, 1031 kgm. m./min. for 120 seconds and 740 kgm. m./min. for 60 seconds at pedalling rates of 88 and 73 R.P.M. respectively. The exercise was as acute as the subject could tolerate. Except for approximately normal findings on the fourth and fifth days, the acidities remained persistently low and were accompanied by abnormally high gastric volumes (graph III, fig. 2). The subject never performed the required work without distress. Relating the rigor of the exercise to the ability of the subject to perform it, this represents the hardest work done in our series. Even under this stringent regime, although suppressed, the stomach showed remarkably good ability to continue its work.

4. *Natural experiences associated with emotional stress.* Excitement, anticipation of unusual events, emotional disturbances and the like derange gastro-intestinal function. Cannon (1909, 1929) has collected much evidence in support of this and the arresting effects of psychic disturbance have been demonstrated under hypnosis (Bennet and Venables, 1920; Luckhardt and Johnston, 1924). McDowall (1927) considers even minor stress of sufficient importance to digestion to make its control and prevention a practical problem in industrial hygiene. However, we observed that in *tube-conditioned subjects*, emotional stress severe enough to induce dryness of the mouth and palpitation, left the acid secreting power of the stomach essentially unaltered. Any athlete who has participated in a tournament knows that the anticipatory excitement is often great even in experienced players. Our efforts to obtain objective evidence of this in terms of a modification of the gastric acidity response to a constant stimulus were also uniformly unsuccessful.

DISCUSSION. The findings suggest that the disappearance of the inhibition of gastric secretory activity which commonly accompanies severe exercise when that muscular activity is repeated is not a training effect in the usual conception of that term. It appears too early to be so classified and once overcome, it is impossible again to induce it even with extreme grades of the exercise to which the subject has become familiarized. Professor Ragsdale of the Psychology Department of this University suggested that the phenomenon might be explained on a basis of "visceral learning." The studies of Coghill (1929) relating behavior to the anatomical development of the nervous system give evidence for the belief that motor skill appears not by grouping simple paths into a complex organization, but by emancipating the appropriate reaction from the initial diffuse response (Coghill, 1930; Herrick, 1931). Individuation may be explained in terms of Fulton's concept of the myotatic reflex (1926). It is every day experience that the initial reaction to a strange situation calls forth a widespread

response and a generalized type of behavior. Just as afferent impulses from key muscles serve to regulate the pyramidal discharge, so the viscera probably report that the sympathico-adrenal mechanism need not be called into play. In the athletic history of our subjects we find a plausible explanation of the rapidity with which the exercise suppression of gastric function disappears. They have been trained to acquire automatized behavior patterns in the realm of neuromuscular skills. They quickly emancipate reactions appropriate to the situation from the initial diffuse response. That the viscera share in this efficiency of regulation is probable, but in the domain of speculation.

The technique of gastric intubation is in itself frequently suppressing to secretory function and an untrained subject cannot be used. The reactions of our subjects may have been conditioned by long laboratory experience so that the very process of intubation restricted the irradiation of impulses, explaining why we failed to demonstrate in any striking way psychic inhibition of acid secretion. Todd and Kuenzel (1929) made fluoroscopic and radiographic studies of the emotional disturbance of gastric patterns and present some evidence in support of this. Fore-knowledge of a contemplated stimulus vitiated the experiment and a psychological stimulus once used could never be employed again.

Cannon (1933) says the changes associated with emotional excitement and vigorous work are similar. Our results suggest that exercise itself is without influence upon the work of the stomach, but that suppression, when it occurs, is due to the irradiation of impulses into the very pathways taken under psychic stress. The mechanism of the emotional inhibition is explained in part by direct action of the sympathetic division of the autonomic nervous system, inhibiting gastro-intestinal muscle tone and the secretion of its glands, and in part by the redistribution of blood which results in splanchnic deprivation and relative anemia of the alimentary tract, thus inhibiting its ability to function (Cannon, 1933). This latter phenomenon is known also to occur in exercise, especially when it is generalized and associated with much sweating. Our findings indicate that when confronted with the necessity of performing strenuous and exhaustive muscular work the beneficent digestive functions are not in abeyance. They carry on in spite of the relative anemia of the organs involved.

It would indeed be disastrous to the efficiency of the gastro-intestinal tract if the vasomotor shifts of exercise were able to inhibit its ability to function under any but the most crucial emergency situations. It requires three or four hours for even a moderate mixed meal to leave the stomach. What goes on in the stomach is of less importance to the organism than the digestive and absorptive functions of the gut. The intestine too must suffer from relative anemia as a result of exercise. During the waking hours the small intestine is probably rarely free of aliment that

must be chemically attacked and then absorbed. Although the stomach is only periodically loaded with foodstuffs, it keeps the intestine much more constantly supplied. To protect the whole alimentary tract from exercise inhibition, muscular work would have to be barred before, after, and between meal times, because when suppression does occur, it outlasts the actual period of effort. It seems to us that the stomach must have what may be called a large margin of safety—the ability to continue working optimally under conditions which seem unfavorable to normal functioning. This thesis should be biologically sound, for processes as fundamental to existence as nutrition cannot be susceptible to too ready change.

CONCLUSIONS

1. The exercise inhibition of the secretion of hydrochloric acid disappears with the repetition of all but extreme grades of work.
2. If the work is so severe that the cardiovascular-respiratory system cannot adjust to it, and it remains always associated with great stress, it continues more or less to inhibit gastric function.
3. The secretory suppression which at first accompanies work under the conditions of our experiments is a part of the total reaction from which a more appropriate response is individuated as the activity becomes automatized.
4. After the response has been conditioned, increasing the severity of the exercise no longer calls forth secretory inhibition.
5. Very severe and exhaustive exercise can therefore be performed without suppressing the acid secreting mechanism of the stomach.

ACKNOWLEDGMENT. Our thanks are due Doctor Tuttle who graciously placed the facilities of the Iowa University Physiology Department at our disposal during the 1934 Mid-West Hockey Tournament which was held in Iowa City. Certain of our observations were made in that laboratory at that time.

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THE EFFECT OF ACUTE ANOXEMIA ON HUNGER, DIGESTIVE CONTRACTIONS AND THE SECRETION OF HYDROCHLORIC ACID IN MAN¹

FRANCES A. HELLEBRANDT, ELIZABETH BROGDON²
AND SARA L. HOOPEs

From the Department of Physiology, University of Wisconsin

Received for publication March 11, 1935

The experiments reported in the preceding paper of this series demonstrated that although at first suppressing, very strenuous exercise may eventually be performed without inhibiting the acid secreting mechanism of the human stomach. Muscular exercise of that severity must be attended by vasomotor adjustments by virtue of which the splanchnic area is subjected to relative anemia. The ability of the acid secreting cells to continue their activity unabated under such seemingly unfavorable circumstances, suggests that the stomach may be an organ relatively insensitive to oxygen want. The work of Van Liere, Crisler and their associates (1930, 1932, 1933, 1934) indicates that such is the case in the dog, for an examination of their published records shows that anoxemia must be exceedingly severe before the digestive movements, hunger contractions or emptying time are significantly affected.

In 1925 Lim, Ivy and McCarthy presented the view that an increase in blood flow will augment the secretion of gastric juice and that all excitatory stimuli act essentially by enhancing the blood flow to the glands. Subsequently Ivy and Farrell (1925) demonstrated that blood supply affects the secretory response of the gastric pouch transplant. In 1927 Lim, Necheles and Ni studied the importance of blood supply to the secretion of the viviperfused stomach and concluded that while increased blood flow may augment secretion already in progress, "gastric secretion occurs within wide limits independently of blood flow." Further, Ivy and Vloedman (1923) found that histamine, which increases the vascularity of the stomach, does not affect its motor conduct, either during periods of hunger or in the interdigestive phase of relative quiescence.

These latter evidences are in agreement with our belief that the splanchnic

¹ This work was made possible in part by a grant from the Wisconsin Alumni Research Foundation.

² From the Department of Physical Education for Women, University of Minnesota.

nic vaso-constriction of exercise in man has little effect upon gastric function. Since the relative anemia of the visceral area may be assumed to result in a diminution of oxygen supply to the stomach, the problem of gastric function during exercise may be indirectly investigated by studying the behavior of the stomach when the organism as a whole is subjected to oxygen want. The object of this inquiry was therefore to determine the effect of anoxemia upon the acid secreting mechanism of the human stomach and upon its ability to maintain adequate fasting and digestive motility.

METHODS. Anoxic anoxia was produced by low oxygen pressure in the inspired air. The subject rebreathed continuously from a closed circuit in which air of the desired composition was made to circulate, carbon dioxide being absorbed by soda lime. Breathing through the nose was prevented by the application of a nose clip. There were no valves in the rubber mouth-piece which was perforated to admit an air-tight connection for the passage of a Rehfuß tube or balloon. A spirometer included in the circuit was equipped with a marking pen to give a graphic record of respiration. The capacity of the spirometer was 8 liters. Preliminary to use in an experiment, the system was filled with room air, circulated and rebreathed to the desired oxygen tension. The subject was then included in the circuit and a finely adjustable stream of oxygen was steadily admitted to replace that removed to meet the metabolic needs of the tissues. As long as the spirometer level remained constant the income of oxygen balanced outgo. If it seemed desirable to reduce further the oxygen tension during the course of an experiment, the inflow valves were shut and the subject removed oxygen from the mixture then present in the system. The small capacity of the reservoir made it possible to reduce quickly oxygen tension by rebreathing. If the anoxemia became too acute, the subject could be revived instantly by increasing the inflow of oxygen. In 1926 Dreyer had described a method like this in principle.

The secretion of HCl was stimulated by standard test meals of oatmeal gruel or 7 per cent alcohol, or by the hypodermic injection of histamine in a dosage of 0.1 mgm./10 kgm. Observations were commenced 14 to 20 hours after the last meal. The contents of the fasting stomach were aspirated and then the secretory stimulant was administered. Saliva was at no time expectorated because the rebreathing apparatus made this impossible during periods of anoxemia. The presence of the mouth-piece of the rebreather made no appreciable difference in this error after the first few trials. Following the administration of gruel or alcohol, 5 to 10 cc. gastric samples were withdrawn at 15 minute intervals, first mixing the contents by aspiration and re-introduction. After a sample had been withdrawn the tube was cleared by admitting a syringeful of air. When histamine was used as the secretory stimulant the total gastric contents

were aspirated quarter hourly. Free and total acidity were determined by titration with $N/10$ NaOH using dimethyl-amino-azobenzene and phenolphthalein as indicators. Results are reported in terms of the amount of $N/10$ NaOH required to neutralize 100 cc. of stomach contents.

Digestive and hunger contractions were studied by recording changes in intragastric pressure, using a small, delicate balloon of condom rubber, a bromoform manometer, and Marey tambour. For the investigation of digestive contractions a meal of 100 cc. of gruel was used. Time was registered synchronously on the kymographs recording intragastric pressure changes and pulmonary ventilation.

After preliminary experimentation during which anoxemia was maintained continuously for as long a time as 109 minutes, it became evident that moderate degrees of oxygen want were without effect upon the phases of gastric function under study. The most intense oxygen want compatible with safety was therefore induced. The duration of the period of anoxemia was limited to one-half hour, and its influence was studied when introduced immediately, 30 and 60 minutes after the administration of gruel, alcohol and histamine. The periods of exposure were never so long when motility was studied because of the shorter and less certain duration of these phases as compared with the acidity cycles. The observations were made on six healthy, young adult women.

The signs of pre-coma anoxic anoxia. Four operators assisted in the conduct of these experiments. The subject sat in a comfortable chair upon an elevated platform, so placed that she might be freely observed in a good light. The director of the experiment sat on a level with the subject and controlled the degree of anoxia by regulation of the oxygen flow. The subject's estimate of her own condition was without value in deciding the limits to which the experiment might be carried. Under acute anoxia critique is conspicuously lacking. The degree of anoxia was judged by close and careful observation of conduct, facial expression, brightness of the eyes, the character of the respiration, color of the skin, mucous membranes and nail beds, and by general clinical signs of distress. Pulse rates were recorded continuously during the anoxemic period. The percentage oxygen in the air circulating in the system was periodically determined.

The subject was maintained on the verge of consciousness. At the detection of unfavorable signs the stream of oxygen was abruptly increased. The subject's condition improved instantly. The promptness of the response to increased oxygen was considered one of the most important indicators of the subject's fitness. She was never held constantly at any given oxygen level. It was adjusted from moment to moment to meet the demands of the organism in toto. The subject was introduced into the circuit when the air contained approximately 10 volumes per cent of oxygen. She was permitted to breathe this until the respirations took on the charac-

ter illustrated in figure 1. The oxygen inflow valves were not until then opened. In a typical half hour experiment the oxygen content oscillated between 7 and 12 per cent depending upon the toleration of the subject. It was enriched above 10 per cent only as necessity demanded and after the revival of the subject was at once carefully reduced again. It was

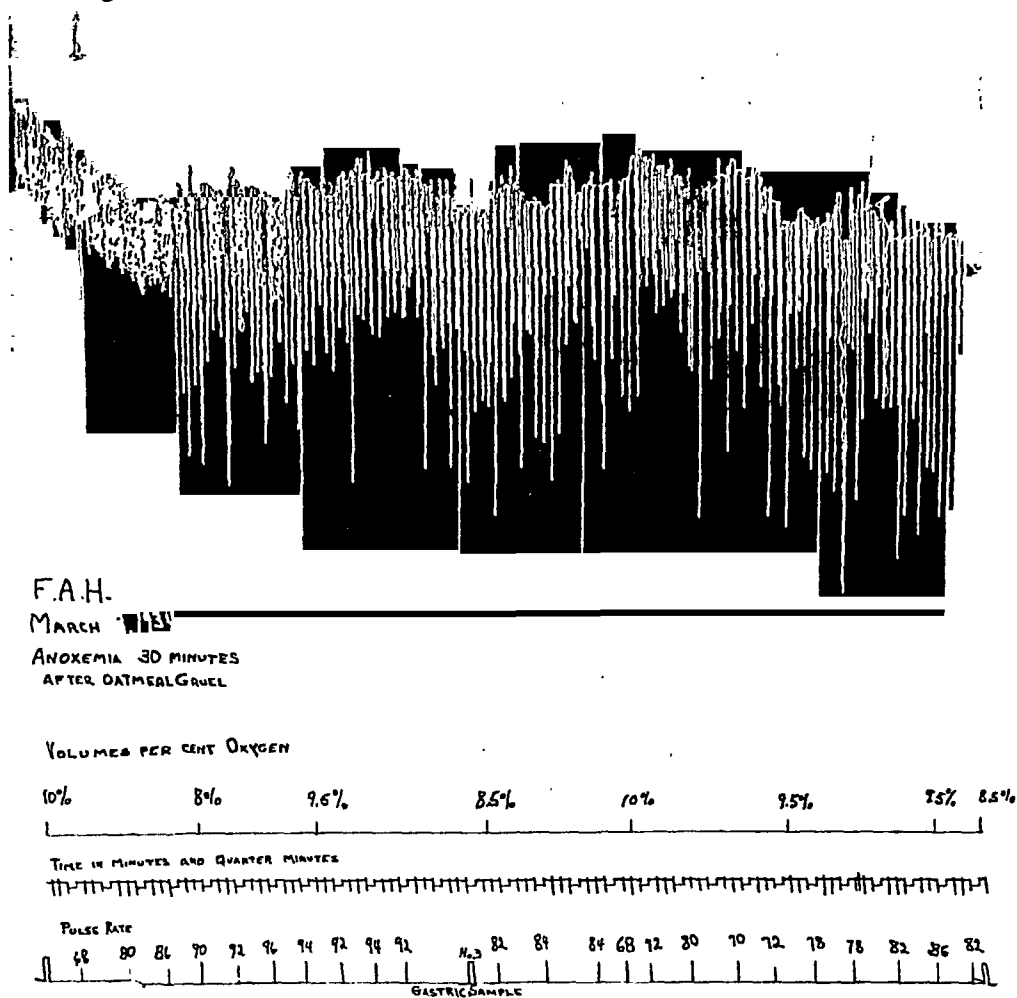


Fig. 1. Kymogram illustrating the respiratory change used as a criterion of pre-coma anoxia.

rarely dropped below 7 per cent and then only momentarily for unconsciousness would have supervened. The character of the breathing, more than any other single factor, was used as a criterion of pre-coma anoxia. The respirations at first increase in depth, less in rate. If the high degree of anoxemia is continued, the respiratory rate soon slows and may be periodic. Breathing is characterized by strikingly deep, gasping inspira-

tions. Expiratory pauses are frequently long, lasting 15 or even 20 seconds. The most profound anoxia in a conscious subject was associated with a respiratory rate of four per minute. Great suppression is illustrated in the kymogram presented. Other constant signs of pre-coma anoxia were uncontrollable restlessness, marked cardiac acceleration and cyanosis.

TABLE 1

The free and total acidity of the gastric juice after the administration of oatmeal gruel, alcohol and histamine to subject F. A. H. under normal conditions and acute pre-coma oxygen want

TIME		OATMEAL GRUEL					ALCOHOL					HISTAMINE							
		Normal		Anoxemia			Normal		Anoxemia			Normal		Anoxemia					
		Mean*	α	Exp. 9	Exp. 8	Exp. 7	Mean*	α	Exp. 4	Exp. 3	Exp. 2	Mean*	α	Exp. 13	Exp. 7	Exp. 12	Exp. 10	Exp. 2	Exp. 11
minutes		cc. N/10 NaOH to neutralize 100 cc. gastric juice																	
15	HCl	6 \pm 5	0	9	0	27 \pm 9	27	42	20	67 \pm 18	76	61	70	73	50	65			
	T. A.	24 \pm 10	18	24	11	44 \pm 14	38	50	29	85 \pm 18	87	75	81	86	67	77			
30	HCl	30 \pm 18	24	23	17	42 \pm 8	40	41	33	98 \pm 11	102	100	92	90	87	72			
	T. A.	48 \pm 19	46	38	27	62 \pm 10	51	52	42	116 \pm 14	111	109	102	100	99	83			
45	HCl	41 \pm 9	23	30	28	42 \pm 10	41	35	43	97 \pm 10	91	100	83	89	93	94			
	T. A.	64 \pm 16	50	42	39	64 \pm 10	52	47	53	113 \pm 12	100	109	92	99	103	102			
60	HCl	44 \pm 5	23	35	35	38 \pm 13	32	55	38	77 \pm 16	67	70	71	88	83	52			
	T. A.	63 \pm 10	44	46	53	64 \pm 15	45	65	47	97 \pm 14	75	80	83	98	99	65			
75	HCl	43 \pm 10	30	32	38	37 \pm 12	25	61	37	68 \pm 17	46	71	49	58	67	50			
	T. A.	64 \pm 12	46	52	52	63 \pm 14	36	72	49	86 \pm 19	56	83	63	70	95	64			
90	HCl	44 \pm 8	27	29	37	33 \pm 10	18	55	35	64 \pm 19	38	44	32	28	38	50			
	T. A.	65 \pm 13	43	44	52	59 \pm 14	33	68	49	77 \pm 22	50	58	48	33	50	65			
105	HCl	43 \pm 13	24	37	34	32 \pm 10	31	57	42										
	T. A.	64 \pm 19	40	49	49	60 \pm 12	45	70	51										
120	HCl	44 \pm 8	30	43	38	34 \pm 8	30	55											
	T. A.	63 \pm 15	45	56	50	59 \pm 6	47	69											

* Arithmetic mean of 8, 21 and 10 experiments respectively.

The acidities of samples obtained during anoxemia appear in italics.

RESULTS. 1. *The influence of anoxemia upon the acidity response to gruel, alcohol and histamine.* These observations were made upon three subjects. To establish a base for comparison, 104 experiments were performed under normal conditions. The variability of the healthy stomach in its secretory response to constant stimuli under well controlled con-

ditions is insufficiently appreciated. Forty-seven experiments were performed to establish the limits of variability in the response to the administration of alcohol, 22 to oatmeal gruel, and 35 to histamine. A total of 66 observations was made under oxygen want. In 38 of these the anoxia was of the pre-coma type. The data on subject F. A. H. is presented in table 1. That obtained on S. L. H. and R. E. B. was in all respects similar. To facilitate the interpretation of the findings the variability was calculated in addition to each subject's average acidity response under normal conditions. Thus one may at a glance compare the anoxemic data in table 1 with that obtained at normal oxygen tensions, and weigh them in terms of the standard deviation or spread in the response which occurs even when all controllable factors are rigorously met.

Observations made during the course of anoxemia approximate normal in a way out of keeping with the profound oxygen want induced. Anoxia was never stimulating during the period of administration. It seemed often to be suppressing, but the findings are impossible to evaluate in a quantitative way because of the relative smallness of the change in comparison with the magnitude of uncontrollable variations. The inhibition is never as great as that described in our first observations of the depressing effects of exercise (Hellebrandt and Hoopes, 1934). The ability of the stomach to secrete a juice of good acid content is relatively unimpaired by anoxemia which widely suppresses activity of the cerebral cortex, and which comes close to paralyzing the vital centers.

Graph I, figure 2, presents the secretory data obtained in the course of the experiment illustrated in figure 1. Pre-coma anoxia was continuously maintained for one-half hour with almost negligible effects on gastric acidity. The suppression induced is just below the limits of normal variability. This typifies the usual response. Depressions as pronounced as that in graph II were only rarely met. The secretory response to gruel seemed in general more readily suppressed than that occasioned by either alcohol or histamine. The comparative susceptibility to inhibition when the anoxic intervals were introduced during early, middle or late phases of the secretory response to stimulation cannot be evaluated with confidence because of the low reproducibility of the results. Subject S. L. H. showed a frequent post-anoxemic acidity augmentation as demonstrated in graph III. This was especially apparent when alcohol was used as the secretory stimulant. Graph IV shows the strikingly competent continuance of the secretion of a juice of high acid content during pre-coma anoxia maintained whilst histamine has its most stimulating effect. We noted not infrequently, and especially in subject F. A. H., that anoxia tended to prolong the interval of high acidity which usually ends abruptly 45 minutes after injection when histamine effects are observed at normal oxygen tensions.

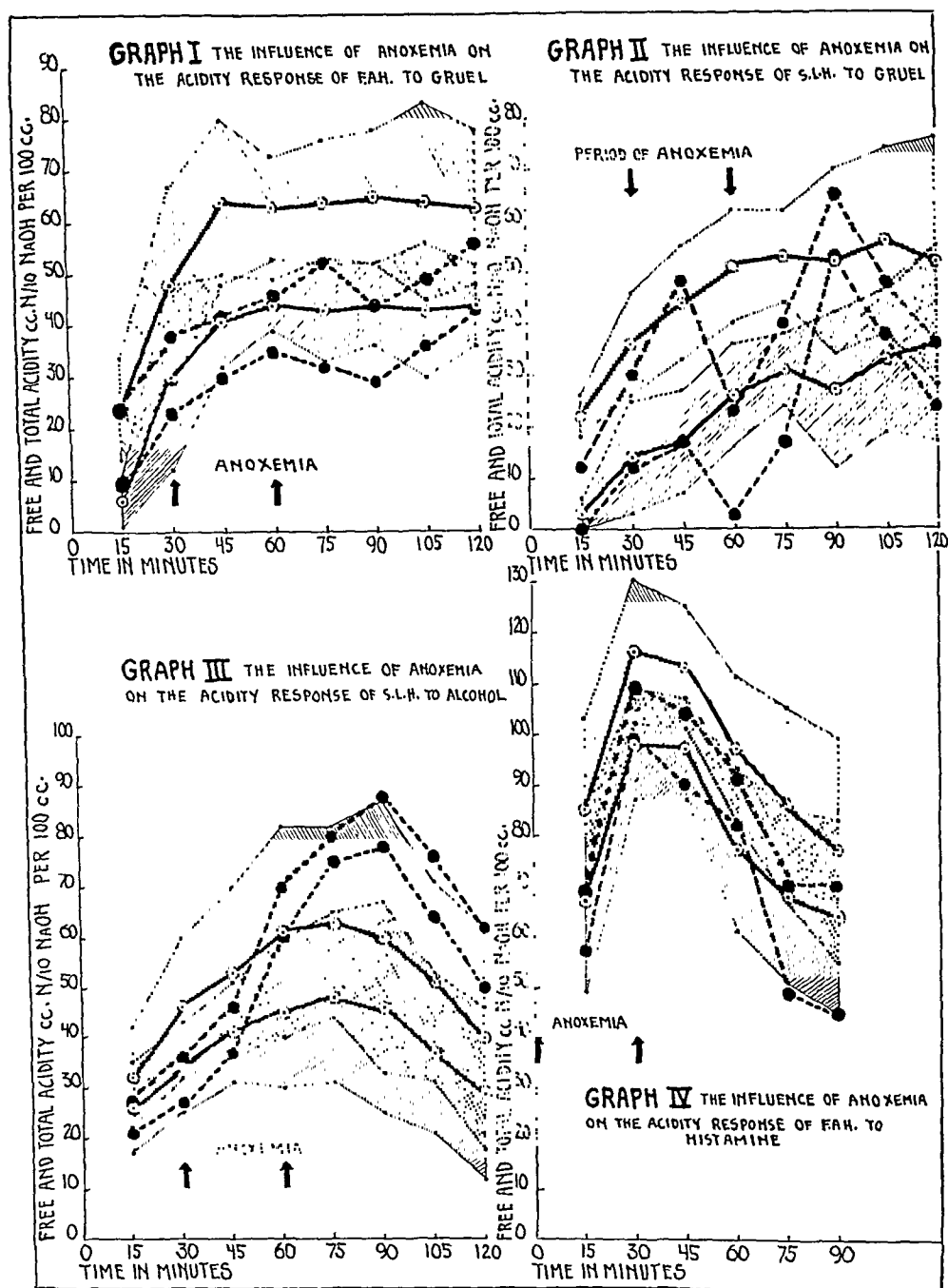


Fig. 2. Graphs showing the variability of the average acidity response under normal conditions (solid lines) and under the influence of pre-coma anoxia (broken lines). The upper lines in all cases represent total acidity, the lower free HCl. The cross hatched areas map out the standard deviation of the mean free and total acidity respectively.

This may be an expression of the same post-anoxic augmentation evidenced in S. L. H. after alcohol.

2. *The influence of anoxemia upon hunger and digestive contractions.* Seventy-one experiments were performed, 33 under normal and 38 under anoxic conditions. Of the latter, 17 were enacted during hunger, 13 during digestion and 8 during the hunger period following a digestive cycle which had itself been subjected to anoxia. There were 3 subjects

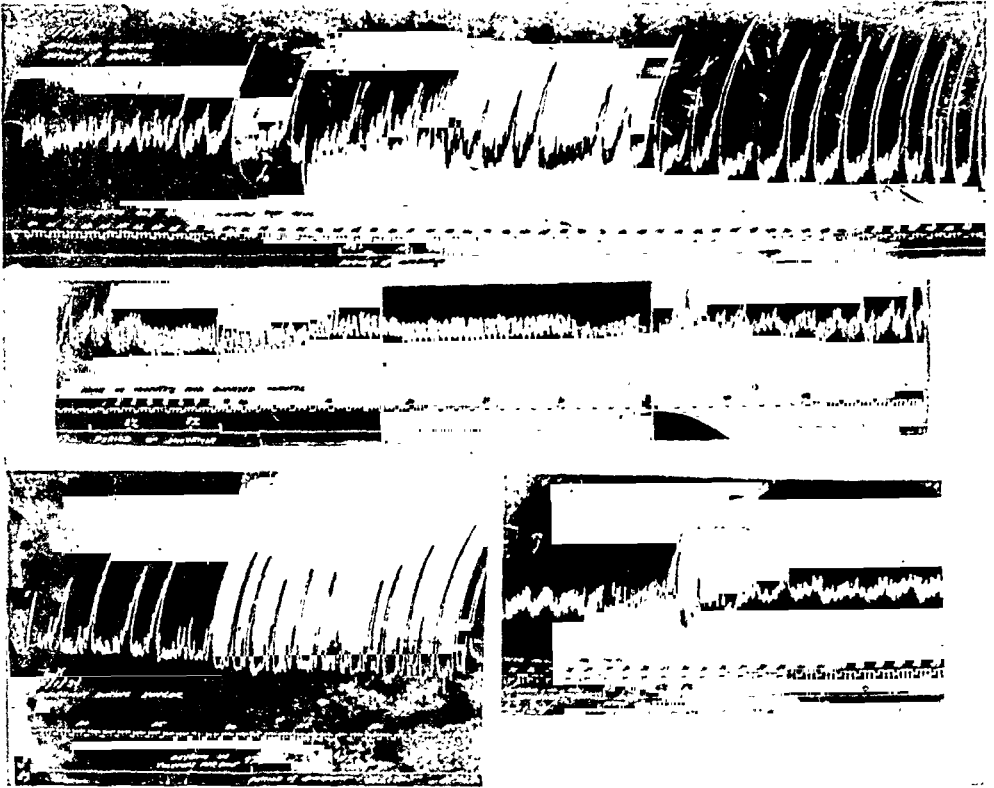


Fig. 3. Kymograms illustrating the effects of pre-coma anoxemia on hunger and digestive contractions.

in addition to those upon whom the secretory studies were made. They contributed 54 of the total observations.

As a whole motility seemed more susceptible to depression than secretion but the degree of change was never marked. We were unable to obtain inhibitions as great as those reproduced in Van Liere's published records on the dog. Examples of the most notable changes induced are presented in figure 3. The effects were for the most part very transitory. Subject C. R. W. was exposed to acute anoxia 9 times. The majority of the observations were made at or below 8 per cent. Because the periods of

exposure were brief, ranging from 5 to 14 minutes and averaging 10, anoxia was pushed to the point of dusky cyanosis. Great air hunger marred the intragastric pressure records. The usual picture was one of gradually developing suppression during anoxia, persisting in augmented form for a few minutes after the return to room air. The average duration of the post-anoxic inhibition was 4.6 minutes in subject C. R. W. She always showed vigorous and prolonged hunger ending in tetanus. As many as 53 hunger contractions of great amplitude occurred during the course of a single hunger period and the longest recorded incomplete tetanus lasted for 13 minutes. When the small meal of gruel was introduced under normal oxygen tension, the tonus rhythm was evident in 20 minutes, well developed in 40, hunger made its appearance in 76 minutes and culminated in tetanus in 120. Anoxemia was introduced three different times during digestion. This insignificantly delayed the reappearance of hunger which occurred at 86, 69 and 107 minutes respectively. Kymogram 1 shows the transitory effect of anoxia imposed during the development of one of these hunger periods following a gruel meal. Hunger had been allowed to develop before the gruel was administered. It terminated in tetanus lasting 8 minutes. The meal was given and in 30 minutes, a sharp, brief exposure to oxygen want. The evanescent effect of this is illustrated in kymogram 4. The tonus rhythm returned and just before these contractions merged into the augmented type characteristic of hunger, the second exposure illustrated in kymogram 1 was made. The disturbance lasted for only a few minutes. Motility of normal amplitude was quickly resumed and typical hunger came on.

J. H. was subjected to anoxia 8 times for intervals ranging between 9 and 14 minutes. The average oxygen percentage was 8. Kymogram 2 shows an unusually prolonged post-anoxemic inhibition, as a result of a 9 minute exposure of a severity which produced a heart rate acceleration from 74 to 102 beats per minute. Motility comparable to that present when anoxia was induced did not recur until after the elapse of 33 minutes. As a result of exposure during which the oxygen fell to 4 per cent and the heart rate jumped to 112, suppression lasted 42 minutes. Evidences of a post-anoxemic augmentation were occasionally seen, although never as clear-cut as those in Van Liere's records. Kymogram 3 illustrates a very moderate augmentation obtained on subject L. H. The anoxemic period was brief but acute for the pulse rate rose from 64 to 116, an increase of 52 beats per minute. The post-anoxemic contractions were of unusual amplitude for the subject concerned.

DISCUSSION. The anoxemia induced in this series of experiments was unquestionably profound. It was of sufficient duration to permit alteration in function if the part concerned is susceptible to oxygen want. We found that cerebral manifestations and cardiac dysfunction occurred be-

fore significant impairment was evident in either secretory or motor behavior of the stomach. This organ is therefore relatively resistant to anoxemia. Though moderately suppressed, it carries on its work in a remarkably competent way under conditions of oxygen want probably rarely exceeded in health or disease. The visceral anoxemia of exercise in which we are chiefly interested, is comparable in duration to that experimentally induced. The findings suggest to us that the paralysis of gastric function which we have observed as an early and transient effect of repeated exercise cannot be due solely to visceral anemia in consequence of the shunting of blood into active muscle and skin regions during the performance of physical work. The findings are in accord with the hypothesis developed in the preceding paper of this series. When suppression of gastric work does occur, it probably finds its chief cause in direct secretory and motor inhibition by way of the sympathetic division of the autonomic nervous system.

CONCLUSIONS

1. Acute anoxemia of the pre-coma type has relatively little inhibiting effect upon the secretion of hydrochloric acid by the normal human stomach, upon hunger contractions or digestive motility.

2. The exercise suppression of gastric function cannot be explained solely by an anoxemic hypothesis. When it occurs, it is probably due to direct secretory and motor inhibition via the thoracic autonomies.

ACKNOWLEDGMENT. Our sincere thanks are due Dr. W. J. Meek for advice in the development of the method and for encouragement during the conduct of these experiments.

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TONUS RHYTHM IN THE ISOLATED GALL BLADDER AND THE EFFECT OF CERTAIN DRUGS

GEORGE M. HIGGINS, KARL DEISSLER AND FRANK C. MANN

From the Division of Experimental Medicine, The Mayo Clinic, Rochester, Minnesota

Received for publication April 15, 1935

Two types of motor activity, which occur in the gall bladders of experimental animals, have been described. One of these is a tonic contraction, which is the result of a sustained muscular activity that produces a marked increase in intravesical pressure, a reduction in size of the vesicle, and the evacuation of its contents. The other is tonus rhythm, which occurs as an alternate contraction and relaxation of the gall bladder with but relatively slight fluctuations in the intravesical pressure.

In our study of the reaction of the gall bladder of sensitized animals to antigen, we observed that tonus rhythm occurred only at certain optimal intravesical pressures. At certain pressures, both the amplitude and frequency of rhythm greatly increased; while at slightly lower or higher pressures, all rhythm was completely abolished. We undertook to study the effect that was produced on tonus rhythm by increasing and reducing the intravesical pressure and to record these changes photographically, with the method hitherto described.

Tonus rhythm of the gall bladder has been studied extensively both in the intact animal and in isolated "in vitro" preparations. Chiray and Pavel, Halpert and Lewis, Ivy and Oldberg, Lieb and McWhorter, Macht, Ravdin and Morrison, Villaret and his associates and Voegtlin and Ivy are but a few of the authors who have studied motor activity in the isolated gall bladder of experimental animals and the manner in which it is affected by the more common drugs. For a complete review of the physiology of the gall bladder the reader is referred to the splendid review by Ivy. We have also studied the effect of certain of the more common drugs on the amplitude and frequency of rhythm in the isolated gall bladder of guinea pigs and dogs. Although these pharmacologic effects are generally known, there are contradictory statements in the literature, and methods of recording and reproducing these effects have not always been satisfactory.

METHODS. Adult guinea pigs and a few dogs were used. The animals were killed by infiltrating the groin with novocain and then severing the femoral artery. The gall bladder and biliary tract were rapidly removed, placed in Tyrode's solution, and a small glass cannula was tied into the

cystic duct. All bile and adjacent hepatic tissue were removed from the preparations, because we have found that they interfere with muscular activity of the vesicle. The gall bladder was immersed in a bath of the Tyrode's physiologic solution (50 cc. for the gall bladders of guinea pigs and 125 cc. for gall bladders of dogs) which was kept at a constant temperature of 38.5°C. Oxygen was supplied freely.

The inlying cannula was connected by means of a rubber tubing with a T-tube, one arm of which was joined to a graduated buret, while the other led to the water manometer, which was arranged to record changes in pressure photographically. We used a manometer of small bore, of the sort ordinarily employed for determining the pressure of the spinal fluid. One centimeter of the manometer contained but 0.2 cc. of fluid, so that slight variations in pressure without significant changes in the volume of the gall bladder were recorded.

When testing for the effect of a drug on tonus rhythm, we withdrew 1 cc. of fluid from the bath, added the drug to this fluid, and then returned it to the bath. In this way, we derived a more uniform dilution of the drug, and the concentration which is indicated is always the concentration in the volume of the bath solution. Every precaution was taken to avoid any accessory influence upon the viscus.

In some experiments a strip of duodenum was suspended in the bath, together with the gall bladder, so that the reactions of duodenum and viscus were recorded simultaneously. A lever, which recorded elapsed time, was fixed in front of the manometer so that its movements were likewise recorded on the revolving sensitized paper.

EXPERIMENTAL OBSERVATIONS. *A. Intravesical pressure and tonus rhythm.* The accompanying illustration (fig. 1) is a photograph of a record of a rhythmically contracting isolated gall bladder of a guinea pig. By means of the buret, the gall bladder had been slowly filled until rhythmic contraction of the viscus began. Rhythm ordinarily began when the intravesical pressure was 25 or 30 mm. of water, although occasionally a gall bladder was encountered which contracted rhythmically when the intravesical pressure was 20 mm., while some did not demonstrate rhythm when this pressure was less than 50 mm. of water. The frequency of rhythm varied in the normal gall bladder from one and a half to two contractions per minute, and the amplitude of rhythm ranged from 30 to 40 mm. Very often we encountered in these gall bladders a secondary rhythm, which was superimposed upon the first, usually at the height or peak of the contraction wave (figs. 1, 10 and 11).

Since tonus rhythm appeared to be dependent on optimal intravesical pressures, we studied the onset of rhythm in relation to a gradually increasing intravesical pressure. We added from the buret to the manometric system, including the isolated empty vesicle at 0 pressure, 0.2 cc.

of fluid every five minutes, and recorded the changes in pressure, which were indicated on the manometer after each addition of fluid. In this manner, we recorded not only the pressure in the vesicle but also the pres-

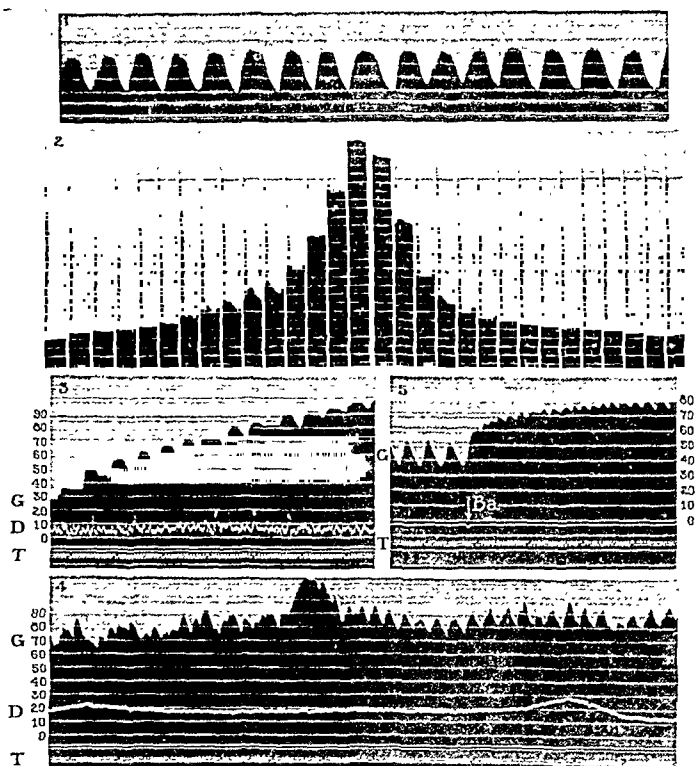


Fig. 1. Record of rhythmically contracting, isolated gall bladder of guinea pig.

Fig. 2. Record of intravesical pressure. The vertical white lines represent five minute intervals between successive additions, to the content of the gall bladder, of 0.2 cc. of fluid. When a pressure of 230 mm. of water had been reached, 0.2 cc. of fluid was withdrawn every five minutes. The intervals between horizontal lines each represent a pressure of 10 mm. of water.

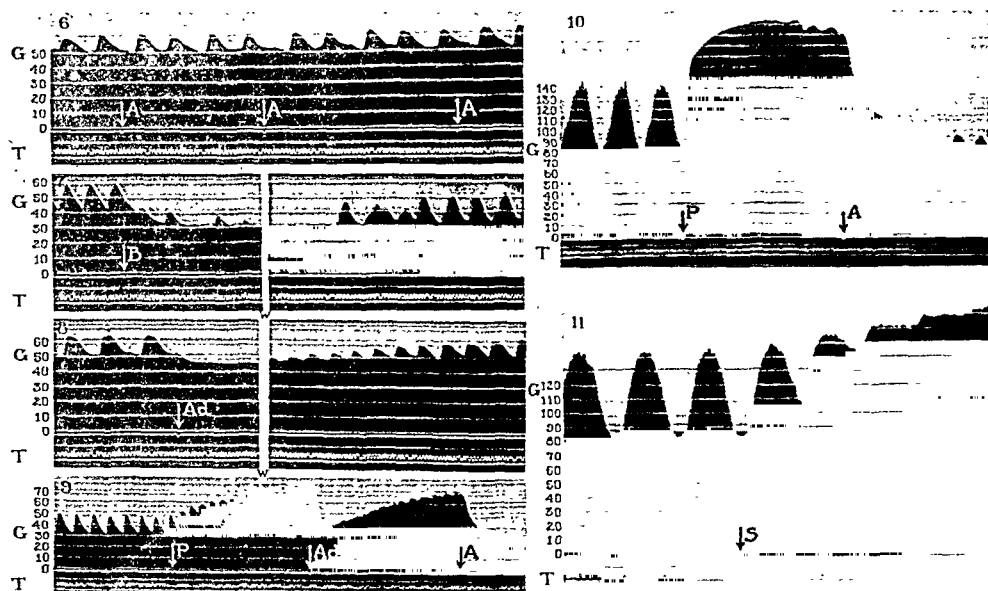
Fig. 3. Spontaneous tonic contraction of gall bladder, of long duration. *G* represents gall bladder; *D*, strip of duodenum and *T*, time marker (interval between broad marks, 1 minute).

Fig. 4. Spontaneous tonic contraction of gall bladder, of short duration; at its height, the complex contraction reaches a pressure of 118 mm. of water. The initials have the same significances as in figure 3.

Fig. 5. The effect, on rhythmic contraction of the gall bladder, of adding barium chloride, 1 to 125,000, to the bath in which the viscus was suspended. *Ba* represents the point in the experiment when the chemical was added. *G* and *T* have the same significance as before.

sure at which tonus rhythm began and when all rhythmic activity ceased. After filling the vesicle to overdistention, a comparable amount of fluid was withdrawn every five minutes and another forty-five second exposure

was made of the manometric fluid level. Fluid was thus withdrawn until the pressure recorded was again zero. Consequently, at the end of the experiment a complete record was available of gall-bladder activity during both the increase and decrease of intravesical pressure (fig. 2). This experiment was repeated a number of times with the same gall bladder and with gall bladders of several guinea pigs, and the results were essentially constant. Under the conditions of our experiment, tonus rhythm began at pressures hovering around 20 to 25 mm. It reached a maximal ampli-



Figs. 6 to 11 inclusive. The effects on rhythmic contraction of the gall bladder, of adding various substances to the bath in which the viscus was suspended. The gall bladders represented in figures 6 to 9 inclusive were from guinea pigs; those represented in figures 10 and 11 were from dogs. In each figure *G* represents gall bladder and *T* time, as before. *W* represents the word "washed", and means that the fluid of the bath was changed twice. The other initials and syllables represent points in the experiments when the various substances were added: *A*, atropine, 1 to 500,000; *B*, autogenous bile, 1 to 750; *Ad*, adrenalin, 1 to 500,000; *P*, pilocarpine, 1 to 500,000; *S*, strophanthin, 1 to 400,000.

tude at pressures from 50 to 70 mm., and usually disappeared or was at a minimum at pressures above 100 mm.

Tonus rhythm, we believe, is more dependent on intravesical pressure than it is on volume, for gall bladders vary in their pressure response to given amounts of fluid. The addition of given amounts of fluid from the buret produced varying amounts of pressure in different gall bladders, but rhythm, however, did not occur until certain pressures were attained. Pressure is dependent on muscle tonus, or on the resistance the vesicle offers to distention. Since muscle tonus in the gall bladder varied in

different animals, and even in the same gall bladder at different times, the resistance to a known volume of fluid likewise varied. Rhythmic activity was remarkably constant, however, when the intravesical pressure ranged between certain limits.

Tonus rhythm occurred as a contraction and relaxation over and above the resistance offered by the vesicle to distention. Ivy and Oldberg observed that rhythmic contractions, which amounted to as much as 2.5 cm. of bile, were superimposed on the tonic contraction. Whenever we added small amounts of fluid to the manometric system from the buret, the gall bladder offered a certain resistance, but when rhythm occurred it appeared over and above the tonus level. When rhythm came to rest, it ceased at those pressures at which it began rather than at the height of its amplitude.

Both the frequency and amplitude of gall-bladder rhythm are dependent on the pressure within the vesicle. When the intravesical pressure was increased, the amplitude of rhythm was invariably decreased, although the frequency of the rhythmic contractions increased. From a frequency of one rhythmic contraction per minute at a pressure of 30 mm., we often encountered ten contractions and relaxations per minute at a pressure of 100 mm. The amplitude of these waves, however, was always appreciably less.

Occasionally, a spontaneous tonic contraction of the gall bladder occurred without any apparent cause and continued for some time without appreciable effect on tonus rhythm (fig. 3). In the case illustrated, nothing had been added to the bath, there was uninterrupted activity of the duodenal strip, and yet the gall-bladder pressure rose from 30 to 115 mm. of water, with but slight change in either the frequency or amplitude of the rhythm. On the other hand, spontaneous tonic contractions may be of short duration (fig. 4) and may completely abolish all rhythmic activity.

B. The effect of certain drugs on tonus rhythm. Although many studies have been made upon the reaction of the musculature of the gall bladder to drugs, we have employed the photographic method to record the effects produced within the viscus.

Histamine had a most potent effect on the gall bladder of the dog and guinea pig. In minute quantities, it produced an almost instantaneous tonic contraction and completely abolished rhythm. In dilute concentration, the intravesical pressure rose from 40 mm. to 200 mm. of water. When the bath to which the histamine was added was changed, and fresh Tyrode's physiologic solution was added, the tonic contraction ceased, relaxation ensued, and tonus rhythm again occurred at the usual pressure level. Following a tonic contraction which was induced by histamine, there invariably was an improved amplitude and frequency of rhythm after changing the fluid in the bath. Ivy and Oldberg observed that a small dose of "cholecystokin" would increase the amplitude of rhythmic contractions.

Atropine, 1 to 500,000 in our hands, was without effect on either the muscle tonus or the activity of a rhythmically contracting gall bladder (fig. 6), although by some observers (5, 6, 7, 8) it is said to cause relaxation of the gall bladder of the cat, dog and monkey. However, when a marked tonic contraction had been induced, the addition of atropine caused a prompt relaxation (figs. 9 and 10).

Conflicting reports of the effect of adrenalin on the isolated or intact gall bladder have been made. Voegtlin and Ivy could not demonstrate any constant effect with the intravenous administration of enormous doses. When we added adrenalin in a concentration of 1 to 500,000 to the solution in the bath, there was a decline of but 5 mm. in the intravesical pressure, although rhythm was completely abolished (fig. 8). Normal muscle tone and gall-bladder rhythm were restored after the preparation was washed and the fluid for the bath was added. During tonic contraction induced by pilocarpine, adrenalin induced a prompt but temporary relaxation of the gall bladder (fig. 9) of the guinea pig, but did not have any effect on the contracting isolated gall bladder of the dog.

Pilocarpine invariably caused a marked contraction of the isolated contracting gall bladder of either the dog or the guinea pig. In a concentration of 1 to 500,000, there was a gradual increase in tonic contraction and a decrease in the amplitude of tonus rhythm (fig. 9). In the dog (fig. 10) there was an increase of 110 mm. in the intravesical pressure, and a complete cessation of all rhythmic activity.

Barium chloride in a concentration of 1 to 125,000 induced an immediate and sustained increase in tonic contraction of the gall bladder, a decrease in the amplitude of rhythm of the contraction, but an increase in its frequency (fig. 5).

Autogenous bile (5) induced a marked effect on muscle tonus and rhythmic activity of the gall bladder. When added to the bath, in a concentration of 1 to 750, there was an immediate decrease of 10 mm. in intravesical pressure, an interruption and a final cessation of all rhythmic activity (fig. 7). Ivy and Oldberg observed no effect on the gall bladder when dilute bile or 1 per cent solutions of bile salts were injected intravenously.

Amorphous strophanthin, in a concentration of 1 to 400,000, had a marked effect on tonus rhythm of the isolated gall bladder of the dog. Intravesical pressure increased approximately 100 mm. of water and all rhythm was essentially abolished (fig. 11).

SUMMARY

Tonus rhythm has been studied in the isolated gall bladder of the dog and the guinea pig. Records of this rhythm have been made photographically on sensitized bromide paper. The isolated gall bladder of the dog,

when suspended in warm Tyrode's physiologic solution, demonstrated rhythmic activity with an amplitude of 60 mm., which ranged from 80 mm. to 140 mm. of water. The isolated gall bladder of the guinea pig demonstrated rhythmic activity with amplitudes ranging from 20 to 40 mm. of water, at pressures which varied from 20 to 50 mm. of water in different animals.

Tonus rhythm is definitely related to intravesical pressure. When fluid was added slowly to the empty gall bladder of the guinea pig, which was suspended in a physiologic solution, tonus rhythm began when the gall bladder pressure was 20 to 25 mm. of water. Rhythm ceased or was at a minimum at pressures of more than 100 mm. of water. Tonus rhythm occurred as a contraction and relaxation over and above the resistance offered by the vesicle to distention.

Histamine produced an almost instantaneous contraction of the gall bladder and completely abolished rhythm. Intravesical pressures rose from 40 mm. to 200 mm. of water. Atropine did not affect either tonus rhythm or the tonic contraction of a rhythmically contracting gall bladder. In the presence of marked tonic contraction, however, atropine induced a prompt relaxation of the gall bladder. Adrenalin, in a concentration of 1 to 500,000, induced a slight decrease in tonic contraction and a cessation of rhythmic activity. Adrenalin induced a prompt but temporary relaxation of the gall bladder during a tonic contraction caused by pilocarpine. Pilocarpine always caused a marked contraction of the isolated gall bladder of either the dog or guinea pig. Barium chloride induced an immediate and sustained increase in the tonic contraction of the gall bladder. Autogenous bile induced a relaxation of the rhythmically active gall bladder with cessation of rhythm. Strophanthin increased tonic concentration and abolished rhythmic activity.

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NEUROGENOUS ACTIVATION OF CILIATED EPITHELIUM

ALFRED M. LUCAS¹

From the Anatomical Laboratory, Washington University School of Medicine, St. Louis²

Received for publication February 1, 1935

The major conclusion that nerves modify the rate of ciliary movement in the palate of the frog, first advanced by McDonald, Leisure and Lenneman (1), has received confirmation by Pohle (2), Seo (3) and Lucas (4), but factual and experimentally derived data reported by each of these later workers has brought into question the validity of some procedures and the conclusions resulting therefrom upon which the original hypothesis was founded. The twofold purpose of the present study has been to seek with the aid of methods more recently developed for study of ciliary activity, support for the major conclusion already advanced and to identify the nerve components initiating the ciliary response.

McDonald, Leisure and Lenneman consider that the cilia of the frog's palate are continuously active, that electrical stimulation of the sympathetic trunk and certain autonomic drugs induce acceleration while stimulation of the brain and the action of certain other drugs slow the rate of movement. Their base line of normal activity determined by the rate of passage of particles across the surface was called into question when the reflection method was developed (5), which method eliminates the use of particles or fluids upon the ciliated surface and which demonstrates that such fluids or particles are, in themselves, stimulating agents and finally that in the frog's palate the unstimulated cilia are completely quiescent.

METHODS. The different phases of the investigation involved the following procedures:

Stimulation of the sympathetic trunk. An exsanguinated unpithed frog fastened to a movable platform, with the jaw retracted, was opened to expose the thoracic sympathetic trunk. Stimulating electrodes on the sympathetic trunk at the level of 1st and 3rd or 4th spinal nerves carried single induction shocks. The intensity of the shock was changed by varying a resistance in the primary circuit.

¹ Aided by the Chemical Foundation, the National Research Council and a grant from the Rockefeller Foundation to Washington University for research in science.

² The preparation of the manuscript was completed in the Department of Zoology, State University of Iowa.

Transection of cranial nerves. The 5th, 7th, 9th and 10th cranial nerves and the cranial sympathetic trunk were severed intracranially as shown in figure 1, after removal of the skull plate. Swabs made on toothpicks aided in the operation and a Graefe's cystotome served as a suitable neurotome. The operations were performed only on one side and the opposite side as a control indicated any extensive injury. Operations on 17 out of 25 frogs were successful. Stimulation of the tongue and the resulting response by the palatal cilia was used to determine the effect of nerve ablation upon the reflex pathway.

Nerve-cilia preparations. The method first devised by Seo (3) was modified to the extent illustrated in figure 2. The tibial nerve of the leg freed of tissue to the level of the foot and the palatine nerve was laid across the stimulating electrodes. The electrode adjacent to the responding tissue was grounded to prevent possible spread of current. A Harvard inductorium whose primary was connected with an interruptor set to give approximately three stimuli per second and with a variable resistance of 1 to 999 ohms produced stimuli of threshold intensities. The amount of resistance served as a measure of stimulating current and threshold values were compared by the values obtained. The threshold responses for the nerve-muscle and nerve-cilia preparations were accepted as the first muscular twitch in the toe of the foot and activation of the palate cilia.

Through the kindness of Prof. George H. Bishop it was determined on the cathode ray oscillograph that the first twitch of the toe or foot muscles corresponds to the threshold of the A potential wave.

Comparison of nerve-fiber size distribution. The palatine and tibial nerves of the bullfrog were prepared for osmification of the myelin by first perfusing with a solution of 0.4 per cent NaCl and 0.5 per cent $MgSO_4$; the resulting edema separates the nerve fibers and this followed by perfusion with $HgCl_2$ saturated in the above salt solution, enhances fixation of the

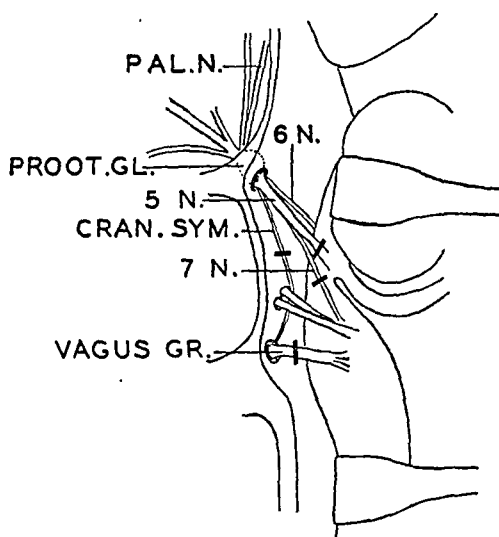


Fig. 1. Cranial and sympathetic nerves. Arrangement of 5th to 10th cranial nerves and cranial sympathetic trunk within the cranial cavity of the common green frog. The 5th, 6th, 7th and sympathetic enter the proötic ganglion from which arises the palatine nerve. The solid lines across the nerves indicate the regions at which they were transected.

fibers in their normal form. The nerves were dissected out, immersed in 2 per cent osmic acid for from 24 to 36 hours and sectioned following double imbedding according to Peterfi. The fiber diameters were measured according to the method of O'Leary, Heinbecker and Bishop (6) and plotted in the manner described by them and by Douglass, Davenport, Heinbecker and Bishop (7).

OBSERVATIONS AND CONCLUSIONS. *Stimulation of the sympathetic trunk.* McDonald, Leisure and Lenneman (1) found that electrical stimulation of the sympathetic trunk accelerated the cilia of the palate and concluded that activator fibers have a pathway through this system.

Repetition of this experiment failed to give these results after a significant possible source of error was taken into account, namely, that the retraction of the jaw brings the afferent nerves of the tongue and adjacent tissues in proximity to the electrodes stimulating the sympathetic trunk.

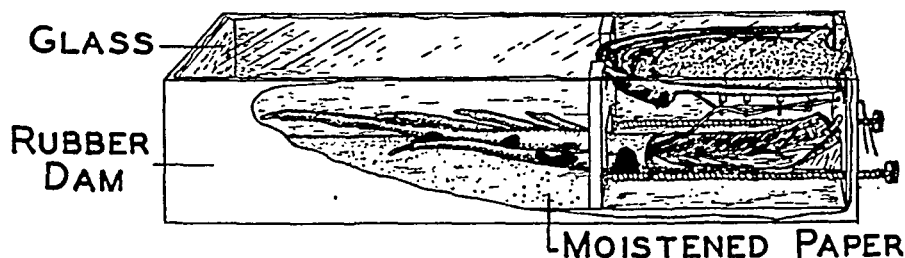


Fig. 2. A sketch of the moist chamber used to obtain values of threshold stimuli to the tibial and palatine nerves. The electrode to the left is grounded, the remaining two carried the electrical stimulus. Wooden pegs were used to fasten the tissues in place.

When such occurs the strength of stimulus adequate to activate the cilia of the palate is about the same as when the palatine nerve is stimulated. The explanation for this result is evident from the work of Seo (3) who demonstrated that a closely integrated reflex exists between points of stimulation on the tongue and correspondingly located points on the palate. Possible error from this source was eliminated by elevating the jaw. This changed the previous apparent threshold value in that the relative current strength required to activate the cilia increased about 10 to 15 times and was about 150 to 225 times greater than that required to produce skeletal muscle contractions by stimulation of the tibial nerve. Moreover, when the shock is made sufficient to activate the cilia, the electrodes and sympathetic nerve being free from adjacent tissue, contractions are induced in the surrounding thoracic muscles, indicating that the spread of current has been apparently responsible for the response and not activation through the sympathetic nerves.

Transection of cranial and sympathetic nerves. The probable absence of accelerator fibers in the sympathetic was subjected to further experimentation utilizing the tongue-cilia reflex described by Seo (3). Two pathways for the reflex are conceivable; one, through the afferent fibers from the tongue to the brain and from there to a sympathetic trunk by way of a spinal nerve, thence to the proötic ganglion and finally along the palatine nerve; two, from the afferent fibers of the brain upward to one of the cranial nerves leading to the proötic ganglion and to the palatine nerve. Seo, unaware of McDonald, Leisure and Lenneman's work, suggested the latter pathway but his experiments in which the nerves were cut outside the cranial cavity did not preclude the first possibility.

The results presented in table 1 based on transections of nerves shown in figure 1, seem to justify the interpretation that no reflex occurs when the 9th nerve is cut because the afferent pathway from the tongue is severed, that the cranial sympathetic trunk is not involved in the reflex pathway

TABLE 1

Effect upon the tongue-palatal cilia reflex of cranial and sympathetic nerve transection

NUMBER OF FROGS	NERVES TRANSECTED	CILIARY RESPONSE ON THE PALATE AFTER STIMULATION OF THE TONGUE
1	9th and 10th cranial	—
1	5th, 7th and cranial sympathetic trunk	—
7	cranial sympathetic trunk	+
1	5th and 7th cranial	—
5	5th cranial	+
2	7th cranial	—

and that the motor afferent neurons to the palatine nerve have their cells of origin in the brain stem and pass peripherally along the seventh cranial nerve.

Cessation of ciliary activity. The reflection method, eliminating the use of stimulating particles or fluids, reveals that the cilia are normally inactive but this does not preclude the possibility that this state is brought about by nerve action. The following facts, however, would indicate that nerves play no part in stopping the cilia but rather that the cessation of movement is an inherent property of these ciliated cells: 1. After a local or a reflex stimulus has passed, the return to the quiescent stage, *in situ*, is gradual, covering a period of several seconds to a minute or more and some cilia come to rest long before others. This is in contrast to the activation response which is known to be initiated by nerves, in that all the cilia, practically simultaneously, within a fraction of a second, attain their maximum velocity. 2. When the palate mucous membrane is removed

and spread, epithelial surface uppermost without a cover glass or the addition of Ringer's solution, the cilia behave in exactly the same way as they do in the intact animal as far as *local* reactions are concerned, that is, they are at rest until stimulated and return to rest some time after the stimulating agent has been removed. The response is, therefore, independent of the central nervous system.

TABLE 2

Ratio of threshold stimuli for nerve-muscle and nerve-cilia preparations

TIBIAL NERVE-FOOT MUSCLE PREPARATION		PALATINE NERVE-CILIA PREPARATION		RATIO OF THRESHOLD VALUES
Number of determinations for each nerve	Average resistance in primary circuit in ohms	Number of determinations for each nerve	Average resistance in primary circuit in ohms	
Green frog*				
2	420	1	31	1 to 13.5
2	447	1	40	1 to 11.2
8	460	8	24	1 to 19.3
13	484	10	26	1 to 18.6
9†	768	10	74	1 to 10.4
5†	600	3	43	1 to 14.0
Average.....				1 to 14.6
Bullfrog‡				
3	317	2	19	1 to 16.7
3	287	2	17	1 to 16.8
2	253	4	19	1 to 13.1
4	332	7	29	1 to 11.4
5	365	11	40	1 to 9.1
1	253	1	14	1 to 18.1
10	224	9	18	1 to 12.4
13	697	9	39	1 to 17.9
6	333	4	20	1 to 16.6
4	402	5	18	1 to 22.3
Average.....				1 to 15.4

* Threshold values obtained separately for palatine and tibial nerves.

† Data for the right and left nerves of the same frog.

‡ Threshold values obtained with both nerves across the electrodes.

Allocation of cilio-accelerator fibers to the autonomic system. Evidence was sought to determine whether the accelerator fibers belong to the somatic motor or autonomic systems by comparing the strength of threshold stimuli applied to the palatine and tibial nerves which would, respectively, elicit ciliary movement and twitching of foot muscles. The moist chamber employed is illustrated in figure 2 and the method already described.

The tibial and palatine nerves were stimulated separately in the first six experiments carried out on the common green frog. The threshold values and their ratio to each other are given in table 2. Possible error due to differences in size of the two nerves is somewhat counter-balanced by the necessary inclusion of more connective tissue with the palatine nerve which tends to make the tissue resistance equal to that of the tibial nerve. Later improvements in procedure involved the use of the bullfrog with its longer palatine nerve and both tibial and palatine nerves were on the electrodes while the value for each was being determined.

In the green frog strength of stimulus to the palatine nerve necessary to activate the cilia averages 14.6 times greater than is required to produce

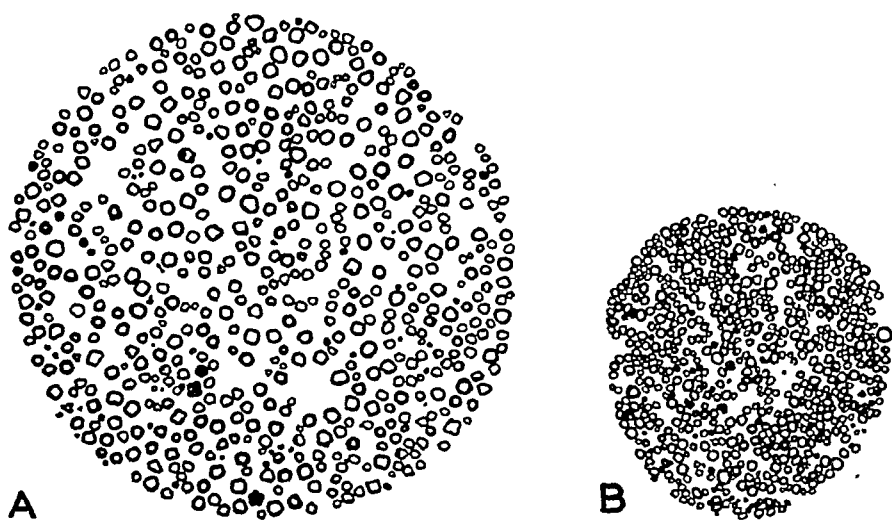


Fig. 3. Magnification of each nerve, $136\times$.³ A. Cross section of tibial nerve of bullfrog; 732 myelinated fibers. B. Cross section of palatine nerve of same frog; 663 myelinated fibers.

slight contractions of the muscles of the foot when the tibial is stimulated. The variation in ratio is from 1-10.4 to 1-19.3. The average ratio for threshold stimuli producing these two responses in the bullfrog is 1-15.4 with variations from 1-9.1 to 1-22.3.

It is on the basis of recent publications by Bishop, Heinbecker and O'Leary (8), and after a reinvestigation of certain relative threshold values given by Heinbecker (9) had been made, that the cilio-accelerator fibers were assigned to the autonomic system. Heinbecker reports for frogs, that to the A potential wave, the B₂ has a threshold about 26 times greater.

³ The edematous and enlarged condition of the tibial nerve in comparison with the palatine nerve is due to the method of preparation and in this case, the latter did not receive as much of the perfused salt solution.

Bishop redetermined the ratio of A and B₂ thresholds in the bullfrog sciatic nerve, in view of the fact that the value of such a ratio depends on the duration of the stimulus, and this in turn depends on the size of the nerve trunk and distance between electrodes, as a part of the resistance of the circuit. Using condenser charges as stimuli, a 0.05 mf. condenser gave a ratio of A to B₂ thresholds in the tibial nerve of the bullfrog in one experiment of 13, in a second of 15, and a 0.02 mf. condenser a ratio of 1 to 20, and with smaller condensers the B₂ wave was not stimulated without unreasonably

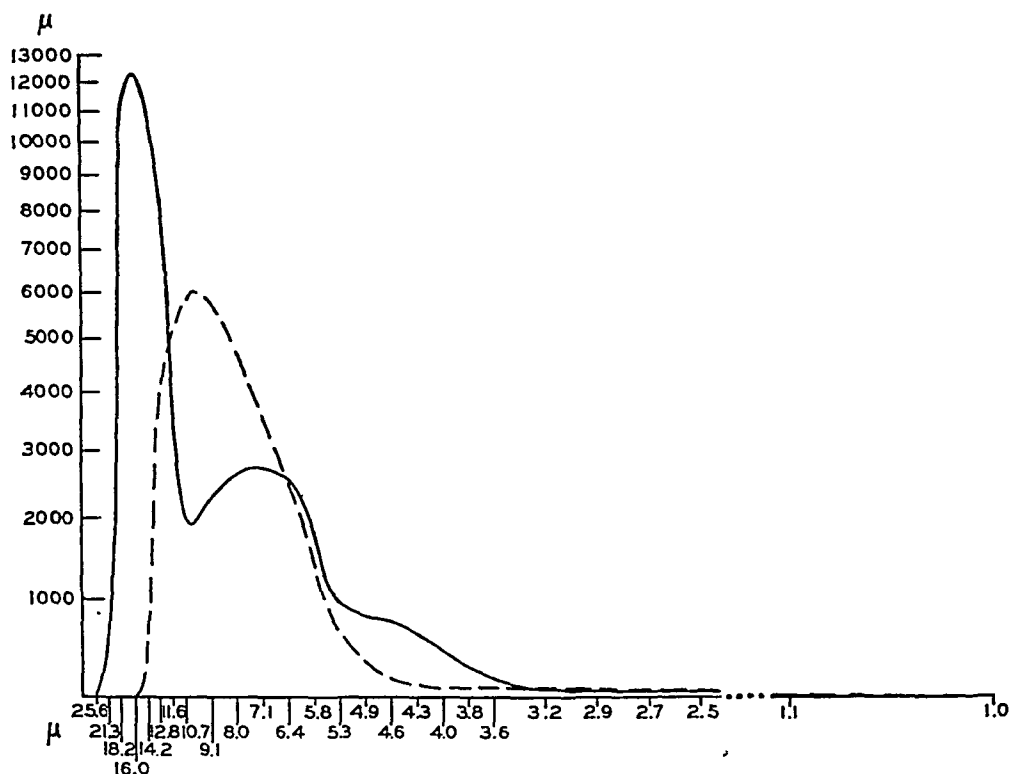


Fig. 4. The distribution of nerve fiber area plotted against a base line of fiber diameter. Tibial nerve is represented by the solid line; the palatine nerve by a broken line.

high voltages. In preparations of the palatine nerve usually employed, where the electrodes were necessarily close together and the resistance correspondingly low, the induction shock must have been prolonged as compared to the usual conditions of sciatic stimulation, which would decrease this ratio. Bishop also determined that the first muscular twitch on the tibial nerve is identical with the threshold for the A potential, making valid a comparison with their results based on oscillographic records.

The threshold ratio of 1:15 for functional responses, falls in the range of 1:13 to 20 obtained for the A:B₂ nerve potentials and on this evidence it is suggested that the cilio-activator fibers belong in the B₂ potential group and on the basis of studies by Bishop, Heinbecker and O'Leary (8), that they belong to the autonomic system rather than to the somatic motor system.

This conclusion, in conjunction with the ones already given would place these fibers in the bulbo-autonomic system having their cells of origin in the brain and coursing through the seventh cranial nerve.

Size distribution of palatine nerve fibers. The palatine nerve is particularly favorable for testing conclusions of Bishop, Heinbecker and O'Leary that the function potential group and fiber size may be correlated. The palate lacks skeletal muscle and presumably contains only sensory fibers and those belonging to the autonomic system innervating ciliated and goblet epithelial cells and blood vessels. The diameters of myelinated fibers in the tibial and palatine nerves illustrated in figure 3 were measured and their areas plotted according to fiber size (fig. 4). The tibial nerve with its somatic motor and sensory components shows a curve with peaks at 16 mu and 7 mu, both of which according to Douglass, Davenport, Heinbecker and Bishop (7) fall within the range of A and B₁ potentials. The sensitive palatal mucosa with its various sensory endings is innervated by fibers falling within a single curve, with a peak at 10.5 mu. That sensory fibers should correspond to a large extent with the second wave of the tibial nerve fibers corroborates the conclusion of Bishop, Heinbecker and O'Leary that many somatic sensory fibers are located in the B₁ or in the last part of the A wave.

The B₂ potential wave for mammalian nerves is correlated with fiber size of 1 to 3.3 mu. Only 10 fibers in the tibial nerve and 30 in the palatine nerve of the bullfrog measure less than 3.6 mu in diameter. In view of the fact that the A:B₂ threshold ratio is less in the frog than in mammals it seems probable that the diameter of fibers belonging to this potential group are larger than in mammals and as represented in figure 4, may include more than the flattened tail for the curve for the two nerves.

The neuro-ciliary relationships which have been discussed are limited to the green frog, *Rana pipiens* and to the bullfrog, *R. catesbiana*. An attempt to extend these observations to turtles and ducks has shown that in the trachea of these animals nerves exert no regulatory influence over ciliary action (Lucas and Douglas, 10).

I wish to thank Prof. George H. Bishop for many helpful suggestions during the progress of these experiments and for his criticism of the manuscript.

SUMMARY

1. Fibers in the palatine nerve of the green frog and the bullfrog are responsible only for activation of ciliary movement and not for its inhibition. The cessation seems to be an autonomous inherent property of these cells.

2. The sympathetic nerve trunk does not carry any of the accelerating fibers to the ciliated epithelium of the palate.

3. The accelerating fibers have their cells of origin in the brain and most of the fibers pass out the 7th cranial nerve.

4. Since the stimulation threshold for the cilio-accelerator fibers corresponds to the threshold for the B₂ potential group of the tibial nerve it is concluded on the basis of work by Bishop, Heinbecker and O'Leary that the cilio-activator fibers belong to the autonomic system and in view of other facts already mentioned that they belong to the bulbo-autonomic or parasympathetic portion of that system.

5. Confirmation is given that the sensory fibers are distributed largely in the B₁ potential group.

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THE INFLUENCE OF PITUITARY GROWTH HORMONE ON THE PHOSPHATASE ACTIVITY OF BONE AND KIDNEY¹

WALTER E. WILKINS, J. ALFRED CALHOUN,² COBB PILCHER AND
EUGENE M. REGEN

*From the Departments of Biochemistry, Anatomy and Surgery, Vanderbilt University
School of Medicine, Nashville, Tennessee*

Received for publication April 3, 1935

Since the pituitary growth hormone and the enzyme phosphatase are thought to be closely associated with bone growth, the question arose as to what influence, if any, daily injection of the hormone would have on the phosphatase activity of the bones.

Phosphatase is known to occur much more abundantly in growing bone than in adult bone. On the other hand, kidney phosphatase is said to increase with age within certain limits. The primary question concerned in this study was whether the renewed bone growth of "weight-plateaued" rats, resulting from the administration of pituitary growth hormone, is accompanied by an increase in the phosphatase activity of the bones. The fact that the epiphyses of the rat do not close with the cessation of growth makes this animal peculiarly well suited for this type of work. Symmetrical skeletal growth, instead of acromegaly, results from the administration of pituitary growth hormone. Presumably the changes in any long bone may be taken as representative of those in the other long bones of the body.

Sixteen adult female rats were used. Before beginning the injections the animals were weighed at regular intervals to insure their being "weight-plateaued". They were then divided into pairs of about equal weight and each pair placed in a separate cage. Of each pair one animal received the growth hormone and the other was used as a control. The experimental animals were injected twice daily for 15 to 32 days with 0.5 cc. of a preparation of anterior pituitary growth hormone. After beginning the injections, all the animals were weighed daily for the first 10 days and three times a week thereafter.

Two animals were sacrificed after 15 days, ten after 26 days, and four after 32 days. The animals were killed with chloroform, the kidneys

¹ The authors wish to acknowledge their indebtedness to E. R. Squibb and Sons for supplying the hormone preparation.

² Fellow in Medicine of the National Research Council.

immediately removed, weighed, extracted with water by the method of one of the authors (1), and phosphatase determinations made according to the method of Jenner and Kay (2). The left femur of each animal was removed intact, thoroughly cleaned, and weighed in a weighing bottle on an

TABLE 1

The effect of repeated subcutaneous injection of pituitary growth hormone on the body, femur and kidney weights, and on the phosphatase activity of the femurs and kidneys

ANIMAL NO.	NO. DAYS INJECTED	WEIGHT AT BEGINNING OF INJECTION	WEIGHT AT SACRIFICE	GAIN OR LOSS IN WEIGHT	WEIGHT OF LEFT FEMUR	PHOSPHATASE ACTIVITY OF FEMUR PER 25 CC. OF EXTRACT (EQUIV. TO 1 GM. OF TISSUE)*	TOTAL PHOSPHATASE ACTIVITY OF ENTIRE WATER EXTRACT OF FEMUR	WEIGHT OF COMBINED KIDNEYS	PHOSPHATASE ACTIVITY OF KIDNEYS PER 25 CC. OF EXTRACT (EQUIV. TO 1 GM. OF TISSUE)*	TOTAL PHOSPHATASE ACTIVITY OF ENTIRE WATER EXTRACT OF KIDNEYS	REMARKS
		grams	grams	grams	grams			grams			
1	Control	272	275	+3	0.719	7.9	5.68	1.957	29	57	Tumors†
2	26	270	317	+47	0.782	6.9	5.42	2.061	25	51	
3	Control	262	256	-6	0.743	6.3	4.68	2.010	37	74	
4	26	272	331	+59	0.760	5.9	4.48	2.394	32	77	
5	Control	266	282	+12							
6	26	257	324	+67	0.743	6.1	4.55	2.147	36	77	
7	Control	236	224	-12	0.733	8.2	6.00	1.948	32	62	
8	26	270	312	+42	0.801	6.3	5.08	2.289	27	62	
9	Control	236	238	+2	0.654	5.9	3.87	2.107	33	70	Tumors†
10	26	251	290	+39	0.713	7.0	5.02	2.259	29	66	
11	Control	248	240	-8	0.769	5.7	4.36	1.949	25	49	
12	15	250	280	+30	0.759	6.1	4.67	2.820	11	31	
13	Control		164		0.427	9.2	3.92	1.187	41	49	
14	32		184		0.498	6.2	3.11	1.629	35	57	
15	Control		177		0.511	8.4	4.28	1.479	35	52	
16	32		214		0.623	6.4	4.02	1.841	37	68	

* These figures represent milligrams of inorganic phosphorus liberated per hour from a substrate of sodium-beta-glycerol phosphate by the enzyme in 25 cc. of tissue extract (from one gram of tissue) at a temperature of 38°C. and maintained at a pH of 8.8 by a glycine-NaOH buffer mixture.

† Nos. 5 and 12 had large tumors in abdominal cavity; probably ovarian cysts or infected uterus.

analytical balance, care being taken to avoid loss of moisture. The extraction and phosphatase determinations were carried out as described by Wilkins and Regen (3).

Of the 16 animals used, two proved to have abdominal tumors (5 and 12)

which made them unfit for the purpose of the experiment; however, as a matter of interest, phosphatase determinations were made on one of these animals (12). As shown in table 1 the injected animals gained from 30 to 67 grams while the controls gained 2 to 3 grams or lost 6 to 12 grams. The left femurs of the injected animals (with the exception of one animal with a tumor, 12) weighed from 2.3 to 22.0 per cent more than their respective controls. The kidneys of the injected animals weighed from 5.3 to 37.2 per cent more than those of the corresponding controls.

With two exceptions (10 and 12), the bones of the injected animals showed a slightly *lower* phosphatase activity per unit of weight than the corresponding controls. The total activity per bone showed more variation; however, it was not appreciably higher in any experimental bone than in its control. This was contrary to the expected findings since it was thought that the phosphatase activity of adult bones would be increased as a result of the renewed growth produced by the growth hormone.

Wilkins and Regen (3, 4) have found that the phosphatase activity of adult as well as growing bone is *decreased* following deep x-ray therapy. The activity of the enzyme may be *increased* in adult bone by certain means. It has been found in this and other laboratories (4, 5, 6, 7) that the enzyme is greatly increased following fracture. This tends to indicate that phosphatase plays a part in bone regeneration. Since phosphatase is thought to play an important part in the growth of young bone, it is not clear why it is not increased in these adult bones which were stimulated to new growth by the hormone. Does this suggest that the mechanism involved in this renewed growth of adult bone is different from that concerned in the growth of young bone?

The phosphatase activity of the kidneys showed the same trend as that of the bones. In each instance, with one exception (16), the phosphatase activity per unit of weight of the kidneys of the injected animals was somewhat lower than that of the corresponding controls. Whether this slight decrease was due directly or indirectly to the hormone, to the slight tissue damage caused by the injections, or to some other factor, we cannot say. It may be of interest to note that the kidneys of 12, one of the tumor animals, showed a very low phosphatase activity.

SUMMARY AND CONCLUSION

Injection of anterior pituitary growth hormone into adult rats was accompanied by an increase in body, bone and kidney weights. The bones and kidneys of the injected animals showed a slightly lower phosphatase activity per unit of weight than those of the corresponding controls. Thus it appears that renewed growth of adult rat bone is not accompanied by an increase in phosphatase activity.

Part of the expense of this investigation was borne by a grant from the Division of Medical Sciences of the Rockefeller Foundation.

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THE PRIMACY OF POLYURIA IN DIABETES INSIPIDUS

CURT P. RICHTER

*From the Henry Phipps Psychiatric Clinic, Johns Hopkins Hospital,
Baltimore, Maryland*

Received for publication March 11, 1935

It is well known that the two clinical symptoms characteristic of diabetes insipidus are polyuria and polydipsia. However, it is not known definitely which of the two is primary, whether the thirst is due to marked water loss by way of the kidney, or whether the increased urinary output follows as a natural physiological consequence of the thirst.

Inasmuch as a definite answer to this question seemed to be of considerable importance for the understanding of the mechanism involved in the production of the diabetes insipidus, it was decided to make a special study of the causal relationship between the polydipsia and polyuria.

The rat was chosen as the experimental animal because diabetes insipidus can be produced in it with fairly consistent success and because its water-intake and urinary output can be measured easily and accurately—advantages which make it possible to procure data from large numbers of animals.

The experiments were performed in two series. In one series urine measurements were made on animals with acute and chronic diabetes insipidus completely deprived of water to determine whether, in the absence of water, polyuria still persists. In the other series an attempt was made to determine which of the two cardinal symptoms, polyuria or polydipsia, appears first after the operation.

METHODS. The operative technique has been described in detail in previous papers (Richter, 1930 and 1934; Richter and Wislocki, 1930). It will be sufficient to state here that diabetes insipidus was produced either by a stab wound with a fine scalpel through the sphenoid bone near the anterior margin of the pituitary gland, or by total or partial removal of the gland by suction applied through a hole in the sphenoid bone.

Animals under observation were kept in small individual cages 7 inches long, $4\frac{1}{2}$ inches wide and $4\frac{1}{2}$ inches deep with a wire mesh bottom. The urine, falling through the mesh to an inclined trough below, was collected in graduated glass cylinders.

RESULTS. *Polyuria of rats with acute diabetes insipidus deprived of all water.* The thirteen rats with acute diabetes insipidus immediately after

operation excreted a large quantity of urine even though deprived of all water. Typical records of a hypophysectomized and a control animal are presented in figure 1 A. The control animal was exposed to the same operative procedure except for the removal of the hypophysis. The urine excretion differed strikingly both in the hourly and total output; the control excreted only 1.7 cc. in twelve hours whereas the experimental animal excreted 25.5 cc. or 8 per cent of its body weight.

The results of this group of experiments are summarized in table 1. The effect of the operative procedure when successfully performed is obvious.

Polyuria of rats with chronic diabetes insipidus deprived of all water. Similar results were obtained with rats with diabetes insipidus of long

TABLE 1

Urine in cubic centimeters excreted during nine hour period immediately following operation by animals deprived of all water

CONTROL RATS		RATS WITH ACUTE DIABETES INSIPIDUS	
Rat 1	1.8	Rat 6	12.7
2	3.3	7	12.1
3	2.5	8	16.6
4	1.7	9	16.5
5	3.2	10	19.4
		11	27.1
		12	19.3
		13	18.4
		14	25.5
		15	19.9
		16	18.4
		17	14.8
		18	24.4
Average.....	2.5		18.8

standing. In a typical record (fig. 1 B) from an animal in which diabetes insipidus had been established sixteen days previously, the output each hour for twelve hours was abnormally high with a total output of 23.8 cc. for the twelve-hour period. For purposes of comparison a typical record is presented of the urine excreted by a normal animal under the same conditions. It can be seen that the hourly output was very much lower than in the experimental animal, and the total output for twelve hours was 3 cc. as compared to 23.8 cc.

The results of these experiments are summarized in table 2 which gives the total urine output for one nine-hour period for twelve normal animals and four animals with diabetes insipidus. The output for the normals varied from 0.7 to 3.4 cc. with an average of 1.5 cc., while the output of the

animals with diabetes insipidus ranged from 4.7 to 23.8 cc. with an average of 10.2 cc. It will be noted further that the output of these animals varied considerably when measured at five to twenty-day intervals; the record for rat 14 of table 2, which is typical, shows readings of 9.0, 8.7, 11.1,

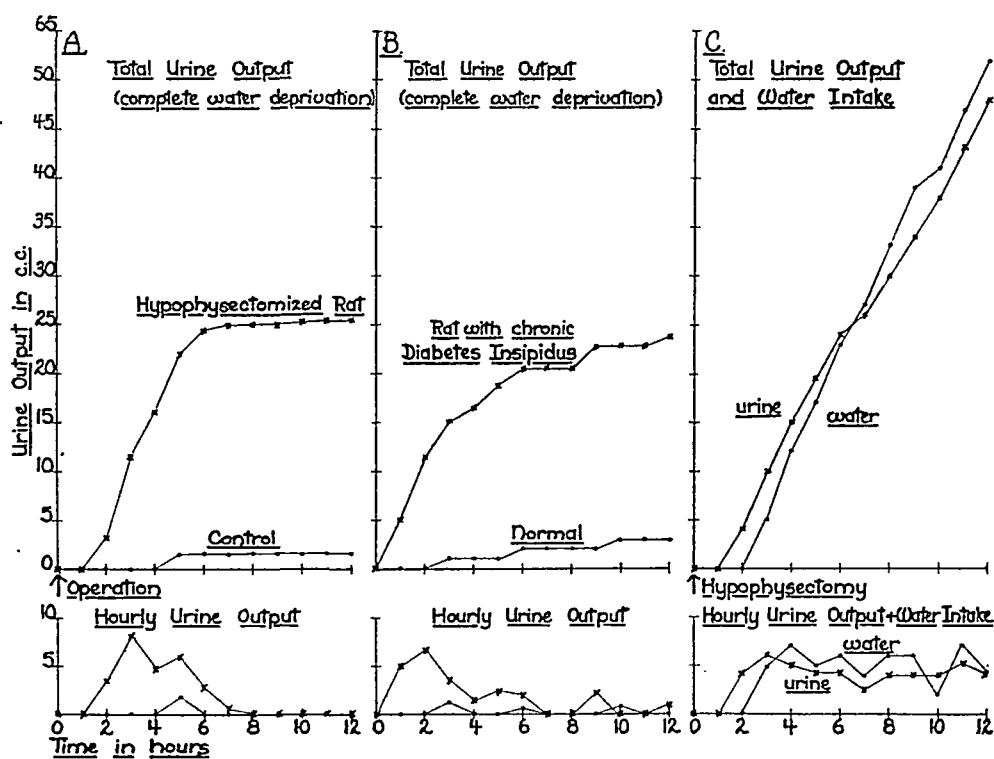


Fig. 1 A. Typical curves of the urine output of an experimental and a control rat, both deprived of water. The experimental animal was hypophysectomized, the control rat was subjected to a similar operation except for the removal of the gland. Urine output is indicated on the ordinates in cubic centimeters.

Upper chart: Total urine output for twelve-hour period.

Lower chart: Hourly urine output.

Fig. 1 B. Typical curves of the urine output of a rat with chronic diabetes insipidus, and a normal rat.

Fig. 1 C. Typical curves showing the onset of polyuria and polydipsia after hypophysectomy. Urine output and water intake are indicated on the ordinates in cubic centimeters.

Upper chart: Total urine output and water-intake for twelve-hour period.

Lower chart: Hourly urine output and water-intake.

and 18.3 cc. on four different days. The basis for these fluctuations is undetermined. They do not seem to be correlated with the degree of polydipsia present as shown in the last column.

It is apparent that animals with chronic as well as acute diabetes insipidus lose large amounts of urine when completely deprived of water.

The priority of the onset of polyuria following hypophysectomy. In these experiments seven hypophysectomized animals were given free access to water so that records were obtained of water-intake as well as urine output. It was found that the polyuria resulting from hypophysectomy preceded the polydipsia by intervals varying from 50 minutes to 1 hour and 50 minutes, with an average of 1 hour and 21 minutes (see table 3). A typical record from one of these animals is presented in figure 1 C in which urine output and water-intake in cubic centimeters are indicated on the ordinates and time in hours on the abscissae. The upper chart records the total amount of urine excreted and the total amount of water consumed during

TABLE 2

Urine output in cubic centimeters per nine hour period of animals deprived of all water

CONTROL RATS		RATS WITH CHRONIC DIABETES INSIPIDUS		
Rat no.	Total urine output per nine hour period	Rat no.	Total urine output per nine hour period	Post-operative average of daily water intake for ten days before intensive experiment
1	1.2	13	23.8	116.9
2	1.1	13	6.3	101.0
3	1.6	13	5.2	90.9
4	3.4	14	9.0	96.7
5	1.6	14	8.7	90.0
6	1.9	14	11.1	101.8
7	2.2	14	18.3	119.5
8	1.0	15	9.6	78.4
9	1.5	15	10.4	67.1
10	0.7	15	9.0	54.5
11	0.8	16	9.0	101.6
12	1.0	16	9.0	92.9
		16	4.7	113.2
		16	8.2	102.9
Average	1.5		10.2	94.8

the twelve hour period following operation; the lower chart shows the urine excretion and water-intake during each one-hour period. It will be noted that at the end of the second hour after hypophysectomy the urine output was 4.8 cc. while the water-intake was still zero. The polydipsia did not start until toward the end of the third hour, so that the polyuria preceded the polydipsia by 1 hour and 25 minutes.

Attention may be drawn here to the fact that, although the onset of polyuria definitely preceded the onset of the polydipsia, the water-intake overtook the urine output by the fourth hour after the hypophysectomy. After that a fairly constant difference of approximately 2 cc. was main-

tained between the water-intake and urine output, the former remaining higher, due partly to the loss of water through channels other than the urine tract such as the lungs and skin, and partly to the loss of urine by evaporation in the collecting troughs.

The results of these experiments support the view that the polyuria is the primary symptom in diabetes insipidus and that the thirst results from the consequent water loss.

DISCUSSION. Observations on the problem of the primacy of polydipsia or polyuria have been made previously, incidental to other experiments on diabetes insipidus. The conflicting results of different workers, however, did not make it possible to draw any definite conclusions. Bailey and Bremer (1921) and Curtis (1924) reported that the polydipsia is primary, while Bourquin (1927) found the polyuria to be primary. The

TABLE 3

Time of onset of polyuria and polydipsia after hypophysectomy

RAT NO.	POLYURIA	POLYDIPSIA	PRECEDENCE OF POLYURIA
1	1 hr., 40 min.	2 hr., 50 min.	1 hr., 10 min.
2	2 hr., 30 min.	4 hr.	1 hr., 30 min.
3	1 hr., 25 min.	2 hr., 40 min.	1 hr., 15 min.
4	3 hr., 10 min.	4 hr., 30 min.	1 hr., 20 min.
5	4 hr., 5 min.	5 hr., 30 min.	1 hr., 25 min.
6	1 hr., 30 min.	3 hr., 20 min.	1 hr., 50 min.
7	4 hr., 25 min.	5 hr., 15 min.	50 min.
Average.....	2 hr., 41 min.	4 hr.	1 hr., 21 min.

results of the present experiments are in agreement with those reported by Bourquin.

The significance of these results as regards the etiology of diabetes insipidus may now be considered. The fact that the polyuria was found to be primary means that the structures injured by the hypophysectomy or stab wound in the hypophyseal region must have some control over the function of the kidney. So far as is known at the present time there are two structures in this region which are reported to participate in kidney regulation: 1, the hypothetical renal center in the hypothalamus; and 2, the posterior lobe (and intermediate lobe) of the hypophysis. All the evidence at hand indicates that nervous impulses from renal centers to the kidneys play little or no part in the regulation of kidney function (Bourquin; Theobald and Verney, 1935; Bailey and Bremer, etc.). There is, however, definite clinical as well as laboratory evidence that the posterior lobe does play an active and important part through the secretion of the antidiuretic pituitrin. (van de Velden, 1913; Gersh, 1934; Burgess, Har-

vey and Marshall, 1933; Cushing, 1933.) Removal, then, of the pituitrin, which normally inhibits kidney function, should cause a diuresis.

It may be noted, in agreement with Greving (1926), that the lesion necessary to produce this result may be located either in the posterior lobe itself (Richter, 1934); in the stalk which contains the fibres from the supra-optic nuclei (Cushing, 1932; Richter, 1933); or in the supra-optic nuclei themselves (Ingram, Fisher and Barris, 1934). The first eliminates the pituitrin at the source, the second and third eliminate it by taking away the stimuli which cause the posterior lobe to produce it.

The one obstacle to the full acceptance of this formulation lies in the fact that the complete removal of the pituitary gland does not always produce diabetes insipidus, and when it does the diabetes insipidus is never permanent. This means that some additional factor must be taken into consideration. In a previous paper it was reported that the anterior lobe plays an important part in the etiology of the syndrome, inasmuch as polyuria or polydipsia are never permanent unless anterior lobe tissue remains (Richter, 1934), possibly due to the presence of a diuretic substance in the anterior lobe. On this basis the diabetes insipidus can be accounted for in terms of a disturbance of the balance between the anti-diuretic pituitrin from the posterior lobe and the diuretic agent from the anterior lobe.

SUMMARY

1. Rats with acute diabetes insipidus produced by hypophysectomy or a stab wound in the floor of the third ventricle excreted large quantities of urine when deprived of water. The average for thirteen rats for a nine-hour period was 18.8 cc. as compared to 2.5 cc. for five rats with a control operation.

2. Rats with chronic diabetes insipidus were found to excrete large quantities of urine even when completely deprived of water. The average for four rats for a nine-hour period was 10.2 cc. as compared to 1.5 cc. for twelve normal rats.

3. The onset of polyuria preceded the onset of polydipsia by an average of 1 hour 21 minutes following the hypophysectomy in seven animals.

4. These experiments indicate that polyuria is the primary factor in the syndrome of diabetes insipidus.

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OBSERVATIONS ON THE LATE EFFECTS OF DENERVATION OF THE CAROTID SINUSES AND SECTION OF THE DEPRESSOR NERVES

M. F. GREEN AND A. DE GROAT

*From the Departments of Physiology and Pathology, University of Arkansas, School of
Medicine*

Received for publication April 8, 1935

Certain of the functions assigned to the carotid sinus and aortic nerves by earlier workers have recently been subjected to critical restudy, chiefly because the original observations were based upon acute experiments and it seemed possible that the observations did not reflect the true functional value of these structures. Particularly in question has been the influence of the sinus and aortic nerves in the regulation of respiration.

In carrying out the studies described below an attempt was made to meet the objections that might be directed toward previous work, such as the non-physiological character of acute experiments, the neglect to denervate the cardio-aortic areas, and the practice of sectioning the depressor nerves in the neck, which in the dog is an uncertain procedure. Three dogs were available which had been completely denervated for 11, 10 and 1 month respectively. The operation consisted of denervation of the carotid sinuses, section of the right cardiac branches of the vagus by the transpleural route and section of the left vagus nerve in the neck. Employing these animals observations were made on the respiratory effects of carbon dioxide, the circulatory effects of amyl nitrite and asphyxia and on the presence of nerve regeneration.

METHODS. The first series of experiments were performed to compare the respiratory reactions of operated and of unoperated dogs to inhalation of increasing concentrations of carbon dioxide. The animals were made to breathe through a mask into a closed system of approximately fifty liters, capacity containing one part of oxygen and four parts of air. Circulation and thorough mixture of the gases was maintained by a rotary pump which was connected through a "Benedict-Roth" metabolism machine with a large reservoir. By means of the tracings made with this machine on a slowly moving kymograph the respiratory rate, amplitude, tidal volume and hence the total ventilation per half minute interval was determined. In making the tracings, the dogs were allowed to breathe for one-half to one minute from the air-oxygen mixture to produce a normal tracing and then

carbon dioxide was run into the system by displacing it from a container with water flowing at a constant rate from the reservoir of the system. By this means the total gas volume was kept constant. The observations were

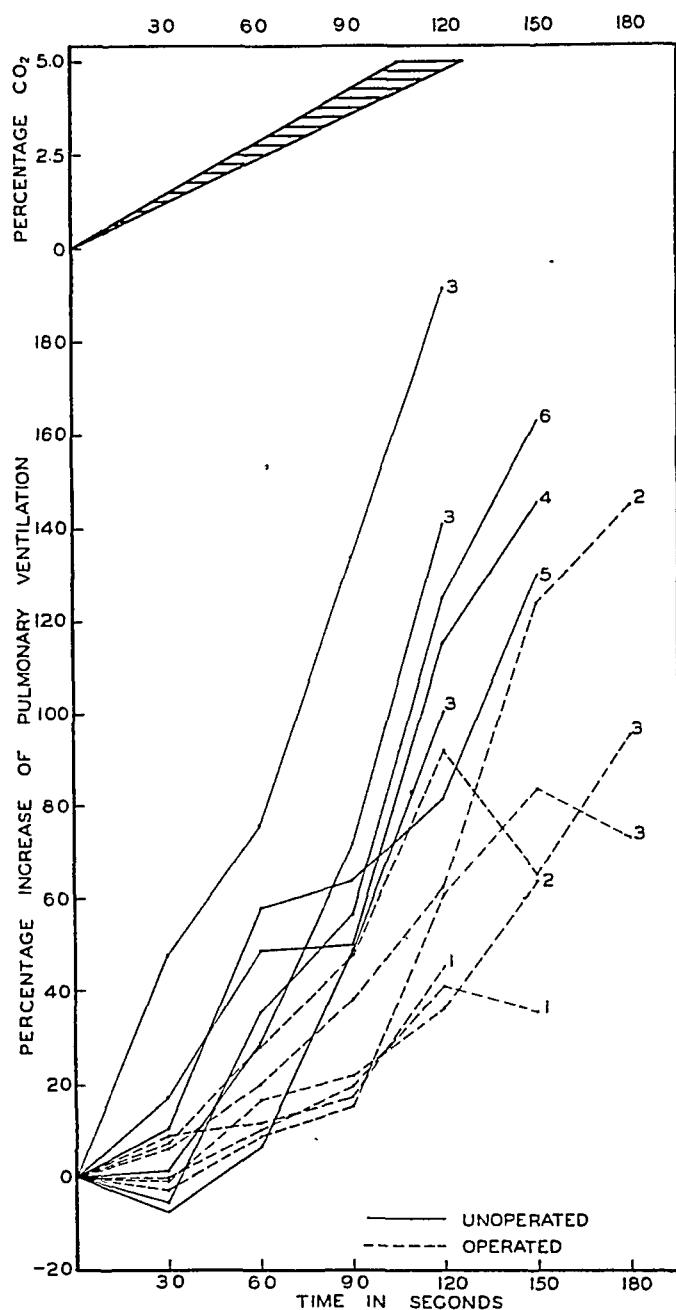


Fig. 1. Showing the percentage increase in pulmonary ventilation during inhalation of increasing concentrations of carbon dioxide. The zero point represents the tidal output per minute when the animal breathed air-oxygen mixture.

carried out at intervals over a period of two months and the most perfect of a large number of tracings were employed in the preparation of the graph.

Finally the animals were lightly anesthetized with ether and a blood pressure tracing taken from the femoral artery. The blood pressure responses to occlusion of both carotid arteries before and after section of the right vagus nerve, to amyl nitrite and to asphyxia were then recorded.

RESULTS. In figure 1 a delay in the response to carbon dioxide in the operated as compared to the unoperated animals is apparent. A comparison of the response in the same animal before and after operation is seen only in dog 3.

The response of the blood pressure in the operated animals to occlusion of the carotid arteries was negligible, there being only the slight mechanical elevation which occurs upon occluding the artery to any large vascular area. This was true both before and after section of the remaining vagus trunk. Section of this nerve had no significant effect upon the heart rate, the increases being respectively 26, 9 and 0 beats per minute in the three animals. Stimulation of the distal end of the right vagus with faradic current gave a normal cardio-inhibitory response.

Inhalation of amyl nitrite produced a fall in blood pressure which was qualitatively normal.

Asphyxia from clamping the trachea caused a moderate rise in blood pressure. The type of curve was practically identical in the three dogs, and the elevation was somewhat less marked than is usually seen following this procedure in animals with intact depressor mechanisms.

In the above experiments we have obtained our results under conditions not previously realized with absolute certainty. Our animals were essentially normal in all respects except that the carotid sinus and cardio-aortic depressor nerves were completely severed. Proof of the functional absence of these nerves was shown in the terminal experiments by the lack of response to occlusion of the common carotid arteries and by the failure of the hypertension and tachycardia to recur following vagotomy. It is an interesting fact that in spite of the extensive operation, some of the cardio-inhibitory fibers persisted. They are assumed to have reached the heart by leaving the vagus trunk with its pulmonary branches which, except for the first one or two, were carefully preserved.

The experiments of Cromer and Ivy (1931), of Gemmill et al. (1933) and of De Groat, Davis and McDonald (1933) seemed to show that under normal conditions denervation of the sinuses was without effect upon respiration. Since these reports appeared, the importance of the sinuses in the respiratory response to anoxemia has been convincingly demonstrated, but experiments with carbon dioxide have been conflicting. Schmidt (1932) reports that the response of anesthetized animals to carbon dioxide is unchanged after denervation of the sinuses. Selladurai and

Wright (1932), however, showed that anesthetized and decerebrate cats with sinuses denervated give a smaller response to inspired carbon dioxide than do unoperated animals. From our own results, which are in substantial agreement with those of Selladurai and Wright, it would appear that the sinuses do have a function in the respiratory response to carbon dioxide, particularly in the low concentrations but that this is not the major factor involved.

That the arterial hypertension which accompanies denervation of the vaso-sensory areas of the sinuses and of the aorta is relatively transient has already been demonstrated (De Groat, Davis and McDonald, 1933; E. Koch, 1934; Heymans and Bouckaert, 1934; Green, De Groat and McDonald, 1935). This result has been attributed to regeneration of the nerves (Koch and Mies, 1929), to the presence of depressor fibers in the vagus other than the main depressor bundle (Goormaghtigh, 1931) and to a lack of sympathetic tonus imposed by the conditions (complete rest) under which the blood pressure is determined (Heymans and Bouckaert, 1934). The functional absence of the carotid sinus and vagus depressor fibers in our animals demonstrates that the failure of the hypertension to maintain itself must be due to compensation by some other mechanism.

Our results do not confirm the statements that denervation of the sinuses reverses the effects of the nitrites on blood pressure (Dautreband, 1934) nor does asphyxia produce a fall in blood pressure. Although the rise in pressure which occurred in our animals was uniformly less than is usually seen, because of the small number of animals and the normal variations which occur in this type of experiment, we are unable to state whether or not there is an altered response.

CONCLUSIONS

1. In three dogs with the sinus and aortic depressor nerves destroyed for periods of 1, 10 and 11 months respectively, a reduction in the degree of respiratory response to carbon dioxide was observed.

2. The failure of the hypertension which follows denervation to be maintained is the result of adaptation rather than regeneration of the carotid sinus and aortic depressor nerves.

3. Destruction of the depressor system is without qualitative effect upon the blood pressure response to amyl nitrite and to asphyxia.

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THE EFFECT OF HYPOPHYSECTOMY AND CEREBRAL MANIPULATION IN THE DOG UPON THE RESPONSE OF THE BLOOD SUGAR AND INORGANIC PHOSPHORUS TO INSULIN¹

I. L. CHAIKOFF, F. L. REICHERT, P. S. LARSON AND M. E. MATHES

From the Division of Physiology, University of California Medical School, Berkeley, and the Laboratory of Experimental Surgery, Stanford University School of Medicine, San Francisco, California

Received for publication February 9, 1935

It has long been recognized that the hypophysis can to some degree control metabolic processes, particularly that of carbohydrates (1, 2, 3). The most striking demonstration of a change in the carbohydrate metabolism of the hypophysectomized animal was found in its abnormal sensitivity to insulin, an observation first made by Houssay and Magenta in 1924 (4), by Geiling, Campbell and Ishikawa in 1927 (5), and since then by many others (6-11). The attempts, however, to determine which part of the gland influences carbohydrate metabolism have yielded conflicting results. The small size of the hypophysis and its inaccessibility render difficult the removal of one lobe without interference with its neighboring lobe or parts. Moreover, because of their proximity to the gland, injury to the tuber cinereum, infundibulum, and hypothalamus may be readily associated with hypophysectomy. It is by no means unlikely that the failure to assess carefully the true damage to the various tissues at the base of the brain accounts, in part at least, for the conflicting views regarding which one of the lobes, when injured or excised, is associated with permanent changes in the carbohydrate metabolism of the organism.

In the course of a study of the metabolism of the hypophysectomized dog, it was deemed advisable to provide so-called "operated controls," i.e., dogs in which all cerebral manipulations up to, but not including, excision of the gland itself were carried out. A study of this preparation as well as of the hypophysectomized animal seemed necessary since the intracranial approach for the removal of the gland involves interference with neighboring tissues in order to bring the gland into full view. It was found that insulin sensitivity could occur in animals in which the hypophysis had been exposed to inspection but still left entirely intact. A study was therefore

¹ The expense of this investigation was defrayed in part by a grant to one of us (I. L. C.) from the Research Board of the University of California, Berkeley.

made of the relation of cranial and cerebral manipulation to insulin sensitivity.

EXPERIMENTAL. Full-grown bitches were used in this investigation. With the exception of those designated by N, which were normal, all dogs were subjected to cranial and cerebral manipulations that varied in degree from an incision of the dura to complete excision of the hypophysis. The animals have been grouped according to the operative procedure to which they were subjected.

TABLE 1

The effect of insulin on blood sugar and inorganic phosphorus of normal dogs

Insulin dose in all cases 1/16 unit per kilo of body weight. Two values only are recorded, (1) postabsorptive, and (2) minimum observed following the insulin injection.

DOG	DATE OF EXPERIMENT	WEIGHT, KGM.	BLOOD SUGAR			BLOOD INORGANIC PHOSPHORUS		
			Preinjection,* mgm. per cent	Minimum		Preinjection,* mgm. per cent	Minimum	
				Mgm. per cent	Time,† min.		Mgm. per cent	Time,† min.
NI. On diet Oct. 30, 1933	Dec. 10	9.5	86.9	80.9	20	3.75	3.34	50
	Feb. 10	10.7	84.5	78.0	110	4.00	3.55	50
	Mar. 3	11.0	83.3	83.3	30	3.52	3.52	30
	May 6	11.8	82.1	77.4	165	3.69	3.39	60
NII. On diet Nov. 15, 1933	Dec. 20	11.2	85.7	76.2	20	3.16	2.67	50
	May 12	13.8	82.1	78.0	105	3.47	3.29	30
NIII. On diet Nov. 22, 1933	Dec. 22	11.2	92.8	88.1	60	3.34	3.07	125
	Feb. 22	12.6	85.7	80.9	20	3.40	3.20	20
	Apr. 21	13.2	75.0	66.7	20			
NIV. On diet Nov. 22, 1933	Feb. 17	12.0	75.0	71.4	80	4.00	3.70	80
	May 9	13.4	73.8	69.7	55			

* Postabsorptive value.

† Minutes after insulin injection.

1. *Craniotomy, retraction of the right temporal lobe, and hypophysectomy* (table 2). The details of this operation have been previously described (12). After the brain had been shrunk by means of an intravenous administration of a concentrated solution of sodium chloride (0.33 gm. per kilo of body weight in a 20 per cent solution), the right temporal muscle was incised parallel to its origin and stripped from the skull. After cranial and dural openings had been made in the area from which the muscle had been removed, the animal was turned on its side, the head rotated so that the vertex was down, the right temporal lobe retracted by means of a spatula

and the hypophyseal region exposed to view. The hypophysis was then removed and the stalk cauterized. *Retraction upon the temporal lobe was*

TABLE 2

The effect of insulin on blood sugar and inorganic phosphorus of dogs in which craniotomy, cerebral retraction and hypophysectomy had been performed

Insulin dose in all cases 1/16 unit per kilo of body weight. Two values only are recorded, (1) postabsorptive and (2) minimum observed following the insulin injection.

DOG	DATE OF EXPERIMENT	WEIGHT, KG.	BLOOD SUGAR			BLOOD INORGANIC PHOSPHORUS		
			Preinjection,* mgm. per cent	Minimum		Preinjection,* mgm. per cent	Minimum	
				Mgm. per cent	Time,† min.		Mgm. per cent	Time,† min.
HI. Operated on Nov. 5, 1933	Dec. 10	11.5	84.5	44.0	95	3.46	1.67	95
	Dec. 22	12.0	75.0	41.7	120	3.07	2.10	185
	Feb. 10	13.6	85.7	47.6	110	2.70	1.82	210
	May 6	15.5	75.0	31.5	60	3.07	1.59	165
HII. Operated on Nov. 10, 1933	Dec. 17	7.5	92.8	52.4	110	4.14	3.43	110
	Feb. 17	8.7	88.1	79.8	60	4.00	4.00	
	May 12	9.8	88.1	79.7	115	4.28	3.06	65
HIII. Operated on Nov. 8, 1933	Dec. 20	9.0	92.8	47.6	50	4.44	2.92	180
	Feb. 17	10.5	78.6	41.0	110	3.80	2.86	175
	May 6	13.0	70.2	57.1	105	3.34	3.00	165
HIV. Operated on Dec. 27, 1933	Feb. 22	10.2	69.0	35.7	105			
	Apr. 21	12.5	80.9	46.9	105	3.08	2.45	105
HV. Operated on Dec. 27, 1933	Feb. 22	8.2	85.7	52.4	100	3.40	3.00	160
	Apr. 21	10.0	83.3	42.9	160	4.90	3.58	100
HVI. Operated on Jan. 3, 1934	Mar. 3†	7.5	42.9	19.0	60	3.52	2.65	60
	May 9§	7.5	38.7	20.2	60	4.00	2.70	60
HVII. Operated on Jan. 3, 1934	Mar. 3	6.9	71.4	35.7	105	2.86	1.75	105
	May 9	7.7	59.5	29.7	180	3.29	2.22	180

* Postabsorptive value.

† Minutes after insulin injection.

‡ Convulsions occurred 75 minutes after insulin injection.

§ Convulsions occurred 85 minutes after insulin injection. This animal ate only one-half of its last meal on the day prior to experiment.

never maintained longer than 2 minutes. Retraction of the temporal lobe for longer than 3 or 4 minutes was found to result in profound postoperative disturbances such as drowsiness, walking in circles, weakness of the left

side, and convulsions. Even death from cerebral edema and increased intracranial pressure may result from too long retraction during this stage of the operation. In the application of the cautery to the base of the brain, care was taken to avoid tissue other than the stalk. The whole procedure from time of incision of the skin to closure of the wound *never occupied more than 20 minutes*. Seven such dogs are recorded in table 2.

In all dogs of this group, a study was made at necropsy of serial sections, 10μ thick, of the whole hypophyseal region. There was a complete ab-

TABLE 3

The effect of insulin on blood sugar and inorganic phosphorus of dogs in which craniotomy and cerebral retraction had been performed

Insulin dose in all cases 1/16 unit per kilo of body weight. Two values only are recorded, (1) postabsorptive and (2) minimum observed following the insulin injection.

DOG	DATE OF EXPERIMENT	WEIGHT, KGM.	BLOOD SUGAR			BLOOD INORGANIC PHOSPHORUS		
			Preinjection,* mgm. per cent	Minimum		Preinjection,* mgm. per cent	Minimum	
				Mgm. per cent	Time,† min.		Mgm. per cent	Time,† min.
CI. Operated on Dec. 4, 1933	Dec. 17	9.0	84.5	54.8	50	3.24	2.79	50
	Dec. 22	9.0	85.7	59.5	50	3.53	2.79	20
	Feb. 10	10.8	83.3	71.4	120	2.67	2.35	120
	May 6	13.3	72.6	51.8	20	2.73	2.19	50
CII. Operated on Dec. 4, 1933	Dec. 20	7.6	80.9	71.4	110	3.53	3.16	50
	Feb. 17	8.7	79.8	75.5	110	3.00	3.00	
	May 9	9.5	72.6	71.4	20	3.00	2.76	20
CIII. Operated on Jan. 3, 1934	Mar. 3	9.5	75.5	50.0	155	3.52	2.90	50
	May 12	12.0	86.9	75.0	70	3.74	3.70	
CIV. Operated on Jan. 25, 1934	Feb. 22	9.8	88.1	50.0	60	3.30	2.80	100
	Apr. 21	10.5	85.7	45.8	160	2.67	1.85	100

* Postabsorptive value.

† Minutes after insulin injection.

sence of anterior, posterior, and intermediate lobes of the gland as well as of all stalk tissue. Small pieces of the pars tuberalis, the largest measuring $1.6 \times 2.0 \times 0.5$ mm., were present at the floor of the third ventricle in dogs HI, HII, HIII and HIV. In the other animals no pars-tuberalis cells were found.

2. *Craniotomy and cerebral retraction* (table 3). In this group of 4 dogs all the operative manipulations up to, but not including, the removal of the hypophysis were carried out. The dogs received the intravenous injection

of the hypertonic solution of sodium chloride. Craniotomy was performed immediately beneath the right temporal muscle after it had been partly freed of its attachment to the skull and the brain exposed by an incision through the dura. A spatula was then inserted and the right temporal lobe of the brain retracted so that the hypophysis and base of the brain were brought into view. *The retraction was maintained for one minute or less* and the wound closed in the usual manner. The whole procedure at no time occupied more than 20 minutes. Thus there was no mechanical interference with the hypophysis during the course of the operation. Inasmuch as hypophysectomy by the intracranial route involves extensive manipulation of the brain before the gland becomes visible for its removal, the dogs in this second group serve as control preparations for the hypophysectomized animals described above.

The brains of these dogs were carefully examined at necropsy for cortical and hypophyseal damage. In addition, serial sections, 10μ thick, were made of the whole hypophyseal region and the condition of the gland carefully studied. A variable degree of adherence between muscle and meninges of the parietal lobe was present at the site of the craniotomy. There was a slight compression of the right lateral ventricle in the 4 dogs of this group but otherwise the brain appeared normal. The microscopic study of the pituitary gland revealed that this structure was completely intact and normal.

3. *Craniotomy and opening of the dura.* The 3 animals recorded in table 4 received the intravenous injection of sodium chloride. Craniotomy was performed beneath the right temporal muscle and the bone removed. The dura was then radially incised; the brain and arachnoid, however, were not disturbed. The wound was then closed in the usual manner.

4. *Normal dogs.* The 4 animals shown in table 1 suffered no operative interferences with cranium or brain.

During their entire stay in the laboratory, the dogs in the 4 groups were maintained under identical environmental and dietary conditions. Each animal received twice daily, at 8:00 a.m. and at 4:00 p.m., a mixture containing 125 grams of lean meat, 40 grams of sucrose and 5 grams of bone ash. In addition, the vitamins were added to this diet twice weekly, vitamin B in the form of a rice-bran concentrate, and vitamins A and D in the form of cod liver oil. With the exception of the first week following the operation, during which time the appetites of these animals were somewhat poor, all dogs, with few exceptions, possessed good appetites, ingesting all food soon after it was served.

In order to compare their sensitivity to insulin, the dogs were injected subcutaneously with $1/16$ unit of the hormone per kilo of body weight, the fall in blood sugar and inorganic phosphorus being used as the index of the animal's response to insulin. The insulin was diluted with 0.9 per cent

sterile salt solution so that the amount to be injected was always contained in 1.0 cc. This dose was adopted, since in a study of the effects of small doses of insulin on the blood sugar of the rabbit, Scott and Dotti (13) found doses smaller than 1/16 unit impractical because of the lack of sufficient precision in blood sugar methods. These workers have also shown that, as judged by the fall in blood sugar, the effects of small doses of insulin in normal animals of varying weights are strictly comparable when the dose is measured in units per kilo of body weight. Thus the minimum dose, the effect of which is experimentally demonstrable in normal animals, has been employed in the present investigation.

The dogs were in the postabsorptive state at the time the insulin was injected, having ingested their last meal 16 hours prior to the removal of the first sample of blood. Blood was taken from the femoral arteries. Eight samples of 5 cc. each were removed from each dog during the course of an experiment. Since the dogs had been previously accustomed to the procedure, most of them showed little, if any, struggle during the removal of blood samples.

Blood sugar was determined with the copper-iodometric reagent of Shaffer and Somogyi (14), while the filtrate was obtained by the precipitation of blood with zinc hydroxide (15). For the determination of inorganic phosphorus Tisdall's method (16) was employed. Whole blood was added, with vigorous shaking, to 5 volumes of 10 per cent trichloroacetic acid and immediately thereafter the mixture was rapidly filtered. As soon as a sufficient volume of filtrate was observed in the flask, 5 cc. were transferred to a 15-cc. centrifuge tube and the inorganic phosphate immediately precipitated with strychnine molybdate. Following the washing and centrifugation of the precipitate, the supernatant fluid was decanted and the tube set aside until the other blood samples had been similarly treated. The filtration and isolation of the inorganic phosphorus of the blood, when carried out with sufficient rapidity, avoid the interference that may result from the hydrolysis of a labile phosphoric ester. All determinations were made in duplicate and the results recorded represent the means of closely agreeing values.

RESULTS. *Normal dogs* (table 1). The postabsorptive values for blood sugar, observed over a period of 5 to 6 months, ranged between 73.8 and 92.8 mgm. per cent, whereas those for inorganic phosphorus lay between 3.16 and 4.00 mgm. In a single experiment no fall in blood sugar followed the injection of 1/16 unit of insulin per kilo, whereas during the course of the 10 other tests the lowest blood sugar values were 5 to 11 per cent below preinjection values. The maximum reduction in blood inorganic phosphorus produced by the same dose of the hormone in 9 experiments was 15 per cent.

Dogs in which craniotomy, retraction of the right temporal lobe, and com-

plete hypophysectomy had been performed. The observations recorded in table 2 were made on 7 dogs over a period of 4 to 6 months following complete hypophysectomy. The postabsorptive blood sugar values in dogs HI-HV ranged between 69.0 and 92.8 mgm. per cent and the inorganic phosphorus between 2.70 and 4.90 mgm. per cent. The lowest blood sugar values were found in dogs HVI and HVII. In the former the 2 values observed were 42.9 and 38.7, whereas in the latter they were 71.4 and 59.5 mgm. per cent.

In HI, which was tested for insulin sensitivity on 4 different occasions, the minimum values found for blood sugar and inorganic phosphorus were

TABLE 4

The effect of insulin on blood sugar and inorganic phosphorus of dogs in which craniotomy and radial incision of the dura had been performed

Insulin dose in all cases 1/16 unit per kilo of body weight. Two values only are recorded, (1) postabsorptive and (2) minimum observed following the insulin injection.

DOG	DATE OF EXPERIMENT	WEIGHT, KGM.	BLOOD SUGAR				BLOOD INORGANIC PHOSPHORUS		
			Preinjection,* mgm. per cent	Minimum		Preinjection,* mgm. per cent	Minimum		Time,† min.
				Mgm. per cent	Time,† min.		Mgm. per cent	Time,† min.	
RI. Operated on July 12, 1934	Sept. 8	13.5	90.5	80.9	60	4.25	3.75	60	
RII. Operated on July 12, 1934	Sept. 8	11.5	76.2	65.5	105	3.77	3.49	60	
RIII. Operated on July 12, 1934	Sept. 8	12.5	78.6	67.8	100	3.68	3.39	60	

* Postabsorptive value.

† Minutes after insulin injection.

respectively 44 to 58 and 32 to 51 per cent below initial values. The most pronounced insulin response in HII occurred in the first experiment after hypophysectomy, at which time the blood sugar showed a maximum reduction of 44 per cent. In the 2 later tests, performed at about 3 and 6 months after the operation, the lowest blood sugar values recorded were 9.5 per cent below the postabsorptive values. In HIII, reductions in blood sugar to the extent of 49, 48, and 19 per cent followed the injection of the hormone. HIV and HV, which were tested for insulin sensitivity at 2 months and again at 4 months after hypophysectomy, showed maximum falls of 36 to 48 per cent in blood sugar. The administration of 1/16 unit of insulin per kilo resulted in convulsions in HVI, while in HVII the

blood sugar fell 36 and 50 per cent on the 2 occasions when its insulin reactions were followed.

Dogs in which craniotomy and retraction of the right temporal lobe had been performed (table 3). In the 4 dogs of this group the postabsorptive values for blood sugar ranged between 72.6 and 88.1 mgm. per cent and for inorganic phosphorus between 2.67 and 3.74 mgm. per cent. CII received insulin injections on 3 occasions and at these times the response to the hormone was no greater than that observed in normal dogs, the maximum fall being to the extent of 12 per cent of the initial value. The 3 other dogs were definitely more sensitive to insulin than the normals. Thus in the 4 experiments carried out on CI over a period of 5 months, the maximum falls in blood sugar were 35, 31, 14 and 29 per cent. Two months after the operation CIII showed a maximum drop of 34 per cent in blood sugar, while 10 weeks later the same dose led to a maximum fall of 14 per cent which, however, was maintained for at least 165 minutes. In the 2 experiments performed on CIV about 1 and 3 months after the operation, the blood sugar was reduced by 43 and 46 per cent respectively.

Dogs in which craniotomy and radial incision of the dura had been performed (table 4). In these animals the blood sugar and inorganic phosphorus were found to be normal, as regards both their postabsorptive values and their reactions to the subcutaneous injection of 1/16 unit of insulin per kilo.

Inasmuch as the 4 groups of dogs received the same diet throughout their stay in the laboratory, variations in their insulin sensitivity cannot be ascribed to differences in caloric ingestion. All dogs were in good nutritional state, and, except for HII and HVII, possessed good appetites, immediately ingesting all food served. HII took several hours to ingest a meal completely, while HVII sometimes failed to consume the whole of one meal by the time the next was served.

DISCUSSION AND SUMMARY

The intracranial approach for the removal of the hypophysis involves 3 distinct manipulations: 1, craniotomy and incision of the dura mater; 2, retraction of the right temporal lobe, and 3, interference in the region of the base of the brain, this last being a procedure that consists in the excision of the gland and in the application of cautery to the stalk. In the present investigation the insulin sensitivity of 3 groups of dogs, in which the various stages had been performed, was compared with the normal. Craniotomy and radial incision of the dura did not alter the sensitivity of dogs to insulin. On the other hand, animals in which all 3 manipulations had been performed, i.e., a complete hypophysectomy, showed a greatly increased sensitivity to the hormone. A dose of insulin, namely, 1/16 unit per kilo, that in normal dogs led to average maximum drops of 5 to 8 per cent in blood sugar and 7 to 10 per cent in blood inorganic phosphorus produced

in the 7 completely hypophysectomized dogs average maximum reductions of 21 to 52 per cent in blood sugar and 16 to 41 per cent in inorganic phosphorus. Although in 2 of these dogs, namely, HII and HIII, the later responses were not as severe as those observed about 1 month after the operation, nevertheless the fact that the same degree of sensitivity was found in HI at 1 month and also at 6 months after the hypophysectomy strongly suggests that the exaggerated insulin reaction is a permanent characteristic of these dogs.

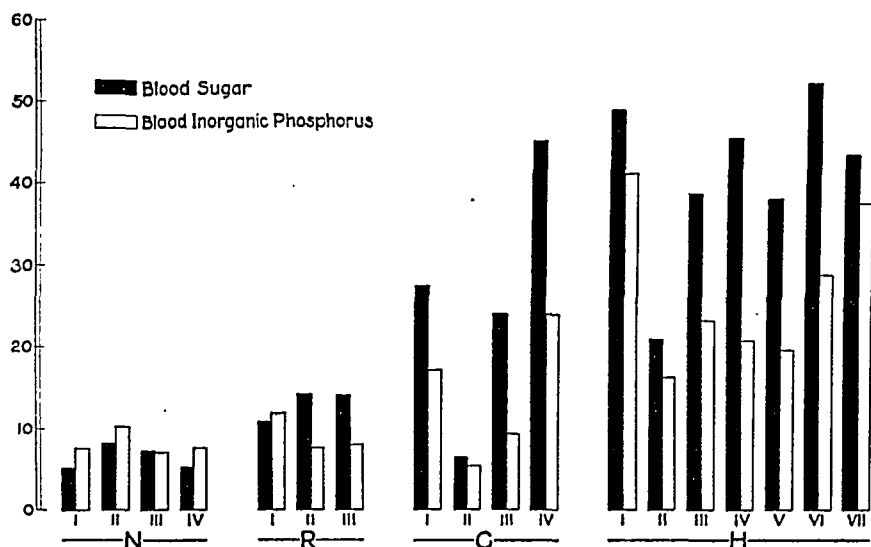


Chart 1. The insulin sensitivity of normal and operated dogs. The scale represents the average of the maximum percentage reductions in blood sugar and inorganic phosphorus produced by the subcutaneous injection of 1/16 unit of insulin per kilo of body weight.

N—normal dogs. *R*—dogs in which craniotomy and radial incision of the dura had been performed. *C*—dogs in which craniotomy, incision of the dura and retraction of the right temporal lobe had been performed. *H*—dogs in which craniotomy, incision of the dura, retraction of the right temporal lobe and complete hypophysectomy had been performed.

Despite the fact that subsequent microscopic study of serial sections of the whole hypophyseal region of all 4 dogs that were subjected to the first 2 manipulations, namely craniotomy and retraction of the right temporal lobe, revealed normal hypophyses, the insulin reaction of only one of these (CII) can be considered entirely normal (chart 1). It is indeed striking that at 3 months after a cranial operation involving no *apparent* mechanical interference with the hypophysis the injection of 1/16 unit of insulin per kilo should have resulted in a drop in blood sugar to the extent of 46 per cent and in inorganic phosphorus of 31 per cent (CIV).

The augmented sensitivity to insulin produced in dogs by craniotomy

and retraction must be the result of the latter manipulation, for dogs in which craniotomy and radial incision of the dura had been performed responded to insulin in a normal manner. Since retraction of the right temporal lobe was involved in the operative removal of the hypophysis, it is necessary to consider the relation of the insulin sensitivity induced by craniotomy and temporal lobe retraction to the sensitivity found after complete hypophysectomy. Chart 1 clearly shows that the degree of sensitivity in the former case was not always as great nor as uniform as in the latter. It seems a reasonable inference, therefore, that part of the increased insulin reaction observed in the dogs hypophysectomized by the intracranial route may be the result of the retraction. On the other hand, although it is not possible in the case of each hypophysectomized dog to determine the extent of the sensitivity conferred by retraction on one of the lobes, it is nevertheless evident that interference in the hypophyseal region cannot be excluded as the major factor in the causation of insulin sensitivity.

The mechanism whereby operative manipulation of the cerebral hemisphere is capable of producing a variable, but nonetheless significant, increase in the degree of sensitivity to insulin is not known at present. As judged by gross and histological examination of the base of the brain, the hypophyses of all dogs subjected to craniotomy and temporal lobe retraction were intact and normal. Some attachment between the meninges of the parietal lobe of the brain and the muscle at the site of the craniotomy had occurred, but it seems unlikely that this could have caused a greater sensitivity to insulin since similar attachments were found after simple craniotomy. Although the cortex of the brain appeared normal and showed normal staining qualities, the right lateral ventricles (operated side) of these 4 dogs were found slightly compressed. It is difficult to relate compression of the ventricles to insulin sensitivity. The fact that insulin sensitivity was most pronounced in dogs that had suffered the greatest damage to the hypophyseal region (excision of the whole gland and cautery of its stalk) suggests that the augmented sensitivity observed in animals after craniotomy and retraction of the temporal lobe was also caused by some interference in this region. Although the pituitary gland itself was not touched, it should be recalled that in this group of dogs retraction was carried as far as the sella turcica so that the gland was exposed to full view. In so doing, it is not unlikely that one or more structures in the region of the hypophysis were injured. The large number of structures, with as yet poorly understood functions, that are present in the small area around the pituitary gland makes it difficult to avoid injury to one tissue while attempting to affect another. Hence identification of the structure or structures responsible for insulin sensitivity can be at present only a matter for speculation.

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EXPERIMENTS ON THE HYPOTHALAMIC NUCLEI IN THE REGULATION OF CHLORIDE AND SUGAR METABOLISM

F. H. LEWY AND F. K. GASSMANN

From the Laboratory of Neurosurgery in the Hospital of the University of Pennsylvania¹

Received for publication February 21, 1935

The fact that lesions of the hypothalamus lead to hyperglycemia and glycosuria and further to polyuria with hyperchloremia was shown by the experiments of Aschner (1912), Camus and Roussy (1922), Leschke (1919), Bailey and Bremer (1921), etc. The question, however, which structures or nuclei of the hypothalamus had to be involved to produce these two effects remained unclarified. It was open to discussion whether the increase of blood sugar was not merely the consequence of handling and tying up of the animal, of narcosis and shock, as Tannenbaum and Hiller (1929) assumed.

The first suggestion of an identification of a hypothalamic cell group connected with the sugar metabolism was made by Brugsch, Dresel and Lewy (1920) when they found that piqure into the dorsal autonomous nucleus of the 10th nerve—followed by glycosuria—led to a retrograde degeneration in the periventricular nucleus. This name was adopted by F. H. Lewy for a cell group situated around the fornix bundle within the hypothalamus. This nucleus was found to be degenerated in a peculiar way in Parkinson's disease in which an alimentary hyperglycemia is mostly present (Dresel and Lewy, 1922).

The hypochloruric polyuria with hyperchloremia seemed to be connected with the paroptic ganglion (Gudden) since F. H. Lewy (1922) in two cases of diabetes insipidus without a pituitary lesion found a marked lesion of this nucleus. The subsequent experimental destruction of the pars posterior of the pituitary body was found to be followed by a retrograde degeneration of the cells of the paroptic ganglion (Kary, 1924).

In order to corroborate these two hypotheses, experiments were made on 33 cats in which either the periventricular nucleus or the paroptic ganglion or both were unilaterally stimulated and subsequently destroyed with the help of the Clarke-Horsley apparatus. Serial histological sections

¹ The experiments were initiated in the Laboratory of the 2nd Medical Clinic of the Charité, Berlin.

through the operated brains showed that in 12 animals the periventricular nucleus, in 6 the paroptic ganglion, in 4 both nuclei were destroyed; 11 animals in which the lesion did not involve either of the two nuclei served as controls.

The publication of our results had to be postponed for a couple of years owing to recent events. In the meantime, the close relationship between the periventricular nucleus and the sugar metabolism has been proved by Loyal Davis, Cleveland and Ingram (1934). We shall, therefore, only refer briefly to this problem.

Penfield's (1934) objections to Davis' experiments are not valid in our case as we were able to approach the nuclei by means of the spherical model of the Clarke apparatus from all planes and sides.

The following protocols illustrate each group of experiments.

TABLE 1

DAY	URINE	SPECIFIC GRAVITY	NaCl	NaCl	BLOOD CHLORIDE	NON PROTEIN UREA	
	cc.		grams per cent	grams per day	mgm. per cent	mgm. per cent	
1	80	1009	0.72	0.57	602	24	
2	70	1015	0.92	0.64	575		
3	85	1012	0.59	0.47	607		
Operation							
4	40	1042	0.198	0.079	635	72	
5							
6	15	1025	0.55	0.082	634		
7	40	1022	1.3*	0.52			

* Eight hours after load with 1.7 gram NaCl in 90 cc. water.

Cat 10. Two thousand three hundred grams. The cat was kept on a constant diet for 8 days and the quantity of output of fluid and NaCl was followed. Pernocton 1 cc. intravenously. Needle inserted laterally on right side and local damage produced. *Next morning* the posterior extremities were spastic, but the animal was active and took fluid and food spontaneously. *Second day* after operation animal was completely anuric. *Third day:* animal drank satisfactorily. Urine normal. *Fourth day* after operation: animal tested by increased NaCl and water load (1.7 gram NaCl in 50 cc. water).

Figure 1 shows the NaCl and sugar level in the blood following the unilateral stimulation and destruction of the paroptic ganglion. Table 1 gives the water and Cl balance in the urine before and after operation, fig. 2 the blood chloride curve with a load of 1.7 gram NaCl before and after operation.

The curves show that the immediate effect of the unilateral stimulation and destruction of the paroptic ganglion is a rise in the blood NaCl from 580 to 670 mgm. per cent lasting longer than 9 hours and levelling finally between 624 and 634 mgm. per cent, i.e., considerably higher than before the operation. There is a hyperchloremia.

Finally, the blood chloride tolerance curve after load with 1.7 gram NaCl is en-

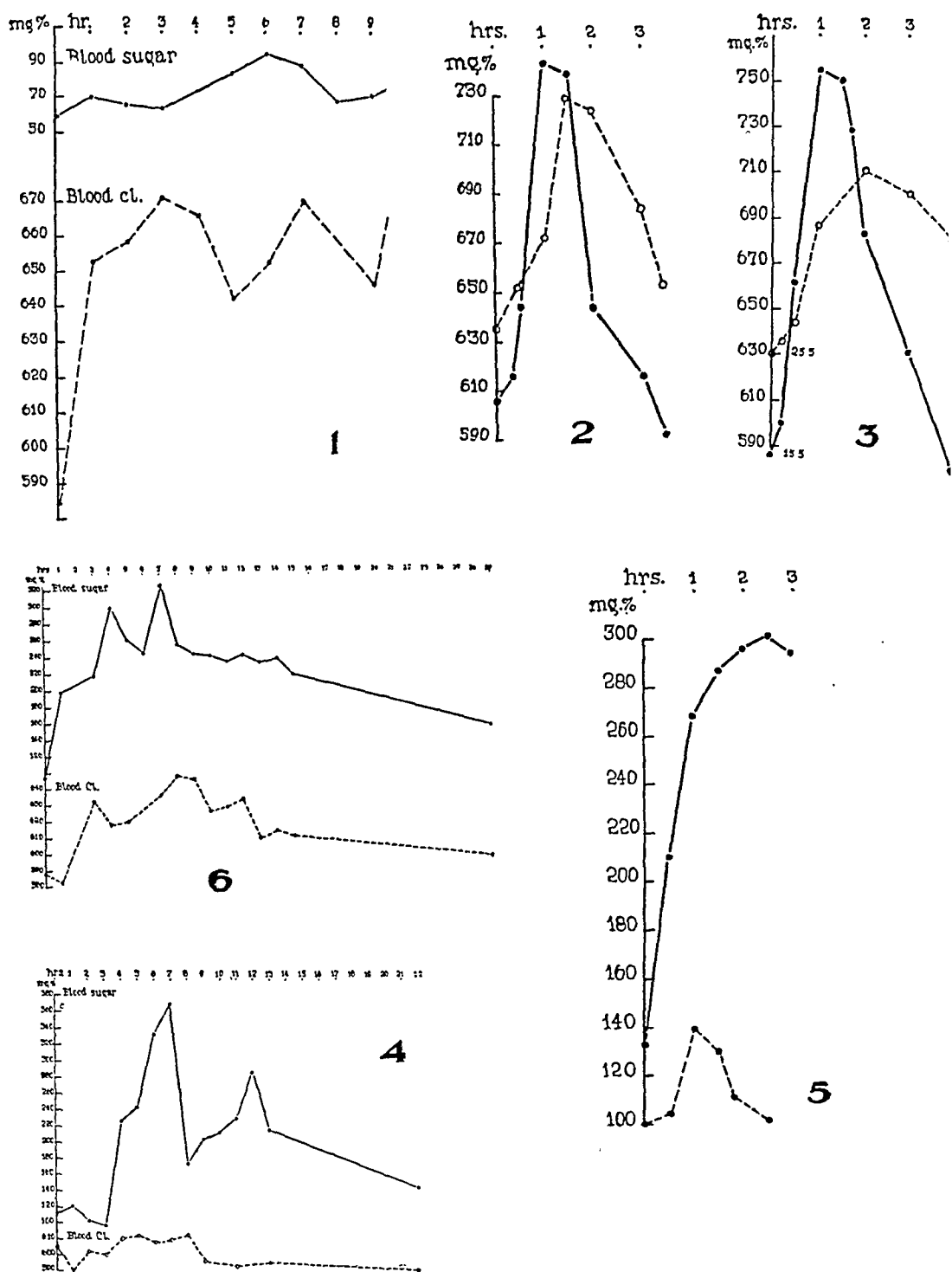


Fig. 1. Cat 10. Blood sugar and blood chlorides after unilateral stimulation and destruction of the paroptic ganglion. Blood sugar not influenced. Increase of the blood chlorides.

tirely different before and after operation. After the operation the curve rises and falls more slowly and the total increase is smaller. The curve, therefore, is not so high and not so steep as in normal conditions.

As this is the most striking point of the present investigations we add in table 2 and figure 3 the chloride-water balance and the tolerance curve of another animal (cat 13) before and after stimulation and destruction of one paroptic ganglion. The figures show the same behavior as in table 1 and figure 2 and the high specific gravity is proof enough that the quantity of urine is credible and that the urine was not lost.

The *lesion* in cat 10 extended over about 1 mm. The lesion was in front of the brain stem next to the ascending fornix column around the chiasm and destroyed the ganglion paropticum. The lesion was nowhere in the neighbourhood of the periventricular nucleus.

To give a clear idea of the entirely different effect of the unilateral stimulation and destruction of the periventricular nucleus we point to figures 4 and 5.

Cat 5. One thousand, nine hundred and fifty grams. Pernocton 1 cc. intravenously. Needle inserted from left lateral aspect and somewhat from above. Destruction with 20 mA 15 seconds.

In the *blood sugar curve* (fig. 4) a maximal increase occurred after an elapse of 7 hours with a second summit 12 hours after operation. In this cat the blood chlorides were normal throughout the whole time of observation. Figure 5 represents the result of a load with 7 grams glucose before and after the operation.

The *lesion* extended over about 1.5 mm. The lesion was in the posterior part of the diencephalon at the superior border of the hypothalamus and covered the periventricular nucleus.

Lastly, cat 9 is an example of a combined lesion of paroptic and periventricular nuclei.

Cat 9. Two thousand, five hundred and fifteen grams. One cubic centimeter pernocton intravenously. Needle inserted nearly horizontally from the left side. The *next day* the cat was sitting, the hind limbs in spastic contraction. The animal in

Fig. 2. Cat 10. Blood chloride tolerance curves with an extra load of 1.7 grams NaCl, before and after unilateral destruction of the paroptic ganglion. Full line before, dotted line after operation.

Fig. 3. Cat 13. Blood chloride tolerance curves with an extra load of 1.7 grams NaCl, before (full line) and after unilateral destruction of the paroptic ganglion (dotted line).

Fig. 4. Cat 5. Blood sugar and blood chlorides after unilateral stimulation and destruction of the periventricular (perifornical) nucleus. Blood chlorides not influenced. Blood sugar increase with a peak at the 7th and at the 12th hour respectively after operation.

Fig. 5. Cat 5. Blood sugar tolerance curve with an extra load of 7 grams glucose, before (dotted line) and after unilateral destruction of the periventricular nucleus (full line).

Fig. 6. Cat 9. Blood sugar and blood chloride curves after unilateral stimulation and destruction of the paroptic ganglion with secondary softening of the hypothalamus involving the periventricular nucleus.

trying to walk fell to the left side. The *second day* the animal kept a compulsory posture on its left side. As we did not succeed in feeding the cat, it was sacrificed.

Examination showed sugar and chlorides increased in the blood 3 hours after operation coming back to normal very slowly in the following 20 hours (fig. 6).

The needle penetrated laterally and reached the paroptic ganglion around the optic tract. The direct lesion was accompanied by a secondary softening of the ipsilateral hypothalamus, pushing the third ventricle to the opposite side and involving the periventricular nucleus.

DISCUSSION. 1. Blood sugar curves as represented in figure 1 are not a rarity. They show that catching and handling of the animals and even electrical stimulation and destruction of an area distant from the periventricular nucleus do not increase blood sugar.

2. Comparison of figures 1 and 4 shows that blood sugar and blood chlorides can vary independently as a consequence of lesions of two distinct cell groups in different regions of the hypothalamus. *A lesion of the paroptic ganglion increases the blood chlorides and not the blood sugar, a lesion of the periventricular nucleus increases the blood sugar and not the blood chlorides.* It is evident that an electrical stimulus can never be exactly limited to a minute area, but the effect is less according to the distance of the lesion from the nucleus.

We can, therefore, entirely corroborate the investigations of Davis, Cleveland and Ingram, although our *modus procedendi* was somewhat different.

3. There is a great difference between the increase of blood sugar and chlorides following *a*, a piqûre of the dorsal vagus nucleus and the reticular substance; *b*, of the cerebellar vermis and *c*, one into the hypothalamic nuclei. Both the form and temporal pattern of the curves differ widely with the place of stimulation.

One may say that the characteristic of *blood chloride and blood sugar curves after lesions of the medulla or the cerebellar vermis* is a quick increase and a gradual decrease within a few hours (Lewy and Shinosaki, 1926; Shinosaki, 1926).

Following a *unilateral lesion of the hypothalamus* the curves look entirely different. The blood chlorides reach the summit of the curve not before the third, often only in the sixth hour after operation. These values are maintained for from 9 to 12 hours after operation or may decrease for a while to increase a second time at the 8th to 10th hour. The normal, as a rule, is not reached before the next day and the level may remain above the normal for a number of days.

Still more characteristic is the delayed type of curve with two distinct peaks in the blood sugar. Here the first maximum is reached, on the average, within 4 to 7 hours. Then follows a marked decrease, although not to the normal and at the 7th to 12th hours after operation a second elevation follows which mostly is not so high as the first. Not until the

day after the operation does the sugar level come back to the normal. Some curves were obtained which showed a rapid initial rise of the blood sugar and successive return to the normal, but we found always that with such curves the seat of the lesion was not within the periventricular nucleus.

Neither the blood chloride nor the blood sugar curves have the smooth decrease which is characteristic for the oblongata, the cerebellar, the adrenalin curves and the curve after a load. Although the general trend is downward there occur irregularities which seem connected in some way with the food intake.

4. The curves with *chloride and sugar load after unilateral hypothalamic lesion* start after the lesion from a higher level, increase and decrease much more slowly. They reach the summit in 2 to 3 hours instead of in 1 hour and are in 3 hours, as a rule, not yet back to the normal. A certain difference is seen in the degree of reaction in that the added chlorides disappear

TABLE 2

DAY	URINE	OUTPUT			INTAKE			BLOOD CHLORIDE
		Specific gravity	NaCl	NaCl	Milk	Meat	Bread	
	cc.		grams per cent	grams per day	cc.	grams	grams	mgm. per cent
1	90	1023	0.55	0.49	200	50	50	583
2	120	1022	0.50	0.60	200	50	50	
3	90	1015	0.53	0.48	200	50	50	585
Operation								
4	25	1052	0.13	0.33				
5	25	1046	0.14	0.034	100	25		
6	30	1048	0.19	0.051	200	50	25	631

after operation more quickly from the blood than in normal conditions while the sugar is changed into glycogen and deposited in the liver more slowly than normally. Thus the sugar curve appears much higher, the chloride curve lower than before the operation but the general form is similar in both of them. The conception of an opposite direction in the time of output of chlorides and sugar after lesion of the central nuclei and tracts is strengthened by the observation of Tokay (1931).

The most important point of our investigations—in our opinion—is the *relation of the chlorides in the blood to the output in the urine and the question of water output after lesion of the paroptic ganglion*. Our results are entirely different from those after the salt-water piqure of Jungmann and Meyer (1913) into the oblongata and from those after unilateral striatum lesions (Tokay, 1931). Veil (1930) considered the derangement of the salt balance to be *hyperchloremic—hypochloruric in unilateral lesions of the hypothalamic centers*; our experiments confirm this.

SUMMARY

1. Unilateral stimulation and subsequent destruction of the paroptic ganglion (Gudden) with help of the stereotaxic apparatus is followed by an increase of the blood chlorides. The chlorides reach the summit of the curve in the 3rd to 6th hour and sometimes attain a second maximum in the 8th to 10th hour after operation. An elevation of the blood chlorides may be present for several days.

The output of chlorides and of urine is distinctly decreased in percentage and in absolute figures.

The blood chloride curve with a load of 1.7 grams NaCl rises and drops considerably more slowly after a lesion of the hypothalamus.

This lesion had no influence upon the blood sugar level.

2. Unilateral stimulation and subsequent destruction of the (perifornical) periventricular nucleus is followed by an increase of the blood sugar. The blood sugar curve reaches its peak at the 4th to 7th hour. Then it drops and has its second maximum within the 7th to 12th hour after operation. The curve returns to the normal from one to three days after operation.

The blood sugar tolerance curve with a load of 7 grams glucose rises and drops considerably more slowly after lesion of the hypothalamus.

This lesion has no influence upon the blood chloride level.

3. Only lesions involving these nuclei have maximal effects on the blood chlorides and the blood sugar.

4. It is open to discussion whether the effect is the consequence of a stimulation or of a temporary paralysis.

5. The impairment of the salt metabolism in hypothalamic lesions is characterized as hyperchloremic—hypochloruric.

6. As these experiments were unilateral and acute they can be directly compared in their results neither with bilateral destruction in animals nor with the chronic human disease of diabetes insipidus.

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ON THE EXISTENCE OF PRO-SECRETIN

V. BROWN SCOTT AND EUGENE U. STILL

From The Department of Physiology, University of Chicago, Chicago, Illinois

Received for publication April 3, 1935

In 1902 Bayliss and Starling published their original researches on the hormone control of the external secretion of the pancreas. They found that acid solutions would extract secretin from the mucosa of dog duodena while neutral solutions would not affect the extraction, at least not nearly so well. For that reason they postulated the existence of a precursor, prosecretin, which was hydrolyzed by acids to yield active secretin.

Since that time there have been many investigations on the secretin problem. In the main, however, they have been directed toward the proof of the physiological part played by secretin and toward the isolation of the hormone. A much smaller number have been concerned with the question of the existence of prosecretin. For that reason many of the published opinions concerning the prohormone have been made from incidental observations, rather than from directed experiments planned to give information concerning its existence or properties. It is only in recent years that the proof of the rôle of secretin in the digestive process has been accepted by the majority of the workers in the field, and it is therefore not surprising that this particular problem has been relatively neglected.

The literature on the subject has been recently reviewed by Still (1931) and although a bibliography is appended to this paper, a review of the literature will not be included here.

In this study we have reopened the question and have attempted to obtain concrete evidence concerning the existence of prosecretin.

EXPERIMENTAL. In order that the work in all the experiments reported in this paper would be comparable, all were of one general plan. Except in special instances, all extracts of the mucosa were made in the ratio 1:20 with respect to the particular solvent used. The insoluble residues were removed by centrifugation, the extracts were neutralized and 5 cc. portions were injected intravenously into dogs prepared for secretin assay in the manner described in the review by Still (1931). In any given experiment, then, a fairly accurate comparison of the activity of the various extracts was obtained and a less accurate comparison of the activity of extracts used in different experiments. A description of the methods

used in preparing the extracts and special points concerning some of the experiments, will be included in the proper place in the text of the paper.

The experiments were planned with the hope of preparing, and possibly purifying, an extract of the duodenal mucosa which would possess certain properties, namely, that the intravenous injection of it would cause no secretion of pancreatic juice until acidified or that the secretagogue potency already present would be greatly increased by acidification.

Series I. Experiments on "untreated mucosa." In these experiments, fresh mucosa was thoroughly ground with sand and extracted with the solvent used. The mixture was centrifuged and the filtrate neutralized, when necessary. The neutralized filtrate was assayed in 5 cc. quantities.

Dog mucosa. A. Fresh dog mucosa was extracted with 0.9 per cent sodium chloride solution, in the manner described above and the extract was divided into two portions "a" and "b." Extract "a" was injected without change or additions to it. Extract "b" was strongly acidified with HCl and after standing for a few minutes an equivalent amount was

TABLE 1

The effect of acidification of neutral saline extracts of fresh duodenal mucosa of the dog

EXTRACT	TREATMENT OF THE MUCOSA	PANCREATIC RESPONSE
		cc.
"a"	Neutral saline extract	0.3
"b"	Neutral extract—acidified	0.2
"c"	Acid extract of the residue	2.2

neutralized and injected for assay. The residue, from the above process, was extracted with 0.4 per cent HCl and centrifuged; the filtrate "c" was neutralized and injected for assay. Table 1 shows the results of one typical experiment.

By reextracting fresh dog mucosa with neutral saline from four to ten times, the quantity of secretin which could be extracted with saline in succeeding extracts rapidly diminished to sub-threshold quantities. The residue, however, upon acid extraction yielded abundant secretin.

When the cellular continuity of the mucosa was destroyed by repeated freezing with CO₂ snow and ether, the results were similar to those already noted except in a few instances in which the saline extract "b" developed slightly more activity upon acidification than was found in the saline extract "a." This can be considered only as mildly suggestive evidence for the existence of prosecretin because the increase was small and such preparations all contained large amounts of noncentrifugable material. Furthermore, the extracts made from the "frozen" mucosa were extremely toxic and it was felt that data from them should be used only as confirmatory evidence.

Similar experiments were carried out in which "frozen" mucosa was dropped into boiling 0.4 per cent HCl or 0.9 per cent NaCl solutions, then cooled and centrifuged. In this way the coagulable protein was removed. The secretin content of these filtrates was found to be in the approximate ratio of 20:1.

Hog mucosa. B. Experiments similar to those described under "A" were conducted, using fresh hog mucosa. The results were essentially the same as the above experiments employing fresh dog mucosa, except for one feature. The amount of saline extractable secretin was more in every instance than that found in the case of dog mucosa. In fact, it was found frequently that almost as much saline extractable as acid extractable secretin was present in the fresh hog mucosa. This difference is not due to differences in freshness of the mucosa and may be due to a species difference.

These results are similar to those of Bayliss and Starling since they show that prosecretin is not soluble in neutral saline. In addition, these experiments show the following: 1. A portion of the secretin is present in the mucosa in an "active" form and may be extracted with neutral saline. The quantity of this saline extractable secretin is variable from animal to animal and there is apparently a greater difference in the relative amounts in different species of animals. 2. After repeated extractions of the "fresh untreated" mucosa with saline, further saline extracts of this mucosa do not contain a threshold quantity of secretin. The residue, however, will yield a considerable quantity of secretin to acid extraction.

It should also be mentioned that reagents other than acids may extract secretin from the fresh mucosa which has been repeatedly washed with saline as described above; such solutions as dilute alkali and aqueous alcohol belong to this group, the alcohol being much more efficient than the dilute alkali.

The preparations just described in this series have contained large quantities of vasodilatin, together with other toxic substances. Attempts were made to "salt-out," with neutral salts, a relatively non-toxic precipitate from slightly alkaline saline suspensions of fresh mucosa. It was hoped that the precipitate would contain not only secretin but its precursor, prosecretin. These experiments were unsuccessful in the latter respect because all of the secretin present in the precipitate could be removed by neutral saline and acidification of the saline extract did not enhance its secretagogue action. Experiments along this line were discontinued.

Series II. Experiments on dried mucosa. The question was raised during the progress of the experiments of series I, concerning the ability of saline to penetrate the cells of the mucosa rapidly enough to extract the secretin. The following experiments were planned with that point in mind. It should be mentioned that Stepp (1912) was probably the first to work

with dried mucosa. His product contained secretin which could be extracted by saline, acids, bile, glycerine and aqueous alcohol. This finding led him to suggest several kinds of secretin.

A. *The "dried mucosa."* Preparations were made by the following procedure. Fresh mucosa of the dog was spread in a thin layer on glass plates and dried in a current of warm air. The dried scales were then milled to pass through a 400 mesh wire sieve.

This preparation yielded only a small amount of secretin on extraction with saline, dilute alkali, and bile salts. However, aqueous alcohol and acids extracted considerable secretin from the dried preparation, the latter extractant being the most efficient. The relative amounts of secretin extracted from the mucosa which had been dried, by these solvents, was of the order obtained from the "untreated fresh mucosa" (series I) treated with the same solvents. It was discovered that the preparation developed considerable acidity during the drying process. The need of a dried preparation which remained neutral or slightly alkaline, was obvious.

B. *"Dried-heated-mucosa."* In order to eliminate as much as possible the development of acidity, the preparation was made in a manner similar to the above (A. "Dried mucosa") except for a preliminary heat treatment of 30 minutes' duration at 80°C., to reduce the effects of enzymes and bacteria.

This product was similar to A ("dried-mucosa") except that more secretin, which was soluble in saline, was present in this mucosa.

C. *"Dried-heated-alkaline-mucosa."* To further eliminate the possible effects of acid development, preparations were made which were kept slightly alkaline (to phenolphthalein) but otherwise prepared as the "heated-dried-mucosa" (B).

This preparation reacted to solvents in a manner similar to the above mentioned preparation.

It should be noted with respect to these preparations that heating causes more of the secretin to become saline soluble. Table 2 shows the results of an experiment in which aliquot samples of the same mucosa were used in making of "dried-mucosa" (A), and "dried-heated-mucosa" (B), of both hog and dog mucosa. The table also indicates the larger amount of saline soluble secretin in the saline extracts.

D. *"Alkaline-crude."* If one extracts the "dried-heated-alkaline-mucosa" (C) repeatedly with saline, the saline soluble secretin can be removed. The residue may be dried with acetone and ether and preserved as a dry powder which possesses the following properties: a. The preparation will yield secretin activity to: 1, dilute mineral acids; 2, stronger organic acids; 3, seventy per cent aqueous alcohol; 4, hot neutral saline. b. The preparation will not yield secretin activity to: 1, NaCl solutions up to 5 per cent; 2, NaOH solutions up to 0.5 per cent; 3, sodium carbonate, 0.2 per cent; 4, solutions of bile salts.

Discussion of series I and II. These experiments show conclusively that the secretin activity of fresh mucosa is not all in the same state, because it is possible, using fresh mucosa (series I) or treated mucosa (series II), to wash out the saline soluble fraction so that further saline extraction fails to yield any activity. However, subsequent extraction of the residue with acids yields a considerable amount of secretin.

Although the experiments in series II are subject to the criticism that proteins of the mucosa have been denatured and possibly otherwise altered by reagents, the experiments of series I are not subject to that criticism.

These results might be interpreted in several ways, among which are: 1. The saline insoluble fraction represents prosecretin. 2. The saline insoluble fraction represents active secretin adsorbed on some of the colloids. 3. The saline soluble fraction represents prosecretin which has been activated by digestive acids but has not been absorbed into the blood stream.

Since we were unable to test the first interpretation and were able to examine the second and third, experiments were planned to that end.

TABLE 2

PREPARATION	ANIMAL	TREATMENT OF MUCOSA	PANCREATIC RESPONSE
			cc.
A	Dog	Unheated	0.2
B	Dog	Heated	1.5
A	Hog	Unheated	1.6
B	Hog	Heated	2.1

Series III. The adsorption of secretin. Recognizing the fact that the product "alkaline crude" (D) may have been denatured in the making, it was, however, selected for these experiments because it contained no saline soluble secretin and further saline extraction would not materially alter its composition. We have assumed the latter to be also the case of fresh, washed mucosa.

Two kinds of experiments were carried out to test the possibility of adsorption.

The secretin in the "alkaline crude" mucosa was inactivated with trypsin. The trypsin was destroyed by heating and the residue tested to show the absence of acid soluble secretin. Purified secretin was then added and the pH adjusted to approximately eight. After stirring for fifteen minutes the solid material was separated by centrifugation and the residue washed repeatedly with neutral saline. The residue was then dried with acetone and ether. The results showed that the powder yielded no secretin to extraction with saline but considerable secretin to acid extraction.

Eight experiments were performed in the following manner: One gram

of the "alkaline crude" preparation was extracted with 20 cc. of 0.4 per cent HCl and the filtrate divided into two portions, "a" and "b." Extract "a" was neutralized and 5 cc. injected intravenously for assay; extract "b" was made slightly alkaline (to phenolphthalein) and allowed to stand at room temperature for 15 minutes before injection. These extracts were prepared to demonstrate the quantity of secretin which the preparation would yield to acid extraction and to determine the effect of weakly alkalizing such a filtrate. One gram of the "alkaline crude" preparation was then extracted with 20 cc. of 0.4 per cent HCl, the pH of the mixture was adjusted to about eight with NaOH and then stirred for 15 minutes. The mixture was then centrifuged and 5 cc. of the filtrate (just alkaline) constituted extract "c." Extract "d" was a 0.4 per cent HCl extract of the residue from "c." Extract "c" was prepared to determine if the adjustment of the pH results in adsorption of secretin. "d" was prepared to determine if the secretin which was adsorbed in "c" and

TABLE 3

Experiment showing the adsorption of secretin on activity "free" mucosa

EXTRACT	NATURE OF EXTRACT	PANCREATIC RESPONSE
		cc.
"a"	Control on "alkaline-crude" preparation to acid extraction	3.2
"b"	Effect of alkali on acid filtrate from the "alkaline-crude" preparation	2.6
"c"	Adsorption of secretin on activity "free" mucosa	0.0
"d"	Acid extract from the residue of "c"	2.8

which was not extractable with neutral saline, could be eluted with an acid solution. Typical results are shown in table 3.

These experiments indicate that in "heated-dried-washed-mucosa" preparations, the residual secretin which is soluble in acids may be adsorbed on the mucosa. This does not prove that secretin is so held in the living mucosa but only indicates that the treated mucosa can adsorb secretin at the proper pH. We are aware of the fact that the previous history of the mucosa may influence considerably such processes.

Series IV. The effect of the exclusion of digestive acids on the state of secretin in the mucosa. Thiry-Vella fistulae were made in ten dogs. The fistulae included the lower duodenum and upper jejunum. The animals were kept for six months or longer during which time the fistulae were frequently flushed with neutral saline. Thus the fistulae did not come in contact with free acids other than those which were formed during the metabolism of the tissues. At the end of the period the animals were sacri-

ficed and the fistulae immediately removed. The mucosa was scraped off and aliquot portions used to prepare extracts for secretin assay. These extracts compared at that time with extracts similarly prepared from normal dog mucosa.

The results were quite typical of those obtained with other fresh mucosae. The solutions which were effective in extracting secretin were, in order of increasing efficiency: saline, dilute alkali, aqueous alcohol and acids. These experiments strongly indicate that the saline extractable fraction of secretin, obtained previously, is not present due to the effect of digestive acids.

SUMMARY

It has been shown that the secretin in "untreated mucosa" exists in two forms with respect to the ability of solutions to extract it, one fraction which is readily extractable with neutral saline, the other which is extractable with acids and is not extractable with saline.

The saline extractable fraction might be termed "free" secretin and the fraction not extractable with saline called "bound" secretin. The "bound" secretin may also be extracted to a lesser degree by weak bases and aqueous alcohol.

We are not in a position to say anything concerning the condition in which "bound" secretin is found in the mucosa. However, we believe the "acid-hydrolysis" hypothesis of Bayliss and Starling not to be entirely tenable, inasmuch as there are many reagents other than acids, such as dilute alkali, aqueous alcohol, etc., which will extract the secretin. Our results only indicate that the "bound" secretin may be held by adsorption to the colloids of the mucosa.

It is not at all improbable that the "bound" secretin is combined with or adsorbed on protein molecules. The reagents which effectively extract it do so by hydrolysis or elution. In that respect "pro-secretin" probably does exist.

Whether or not the "free" and liberated "bound" secretin are the same substance is undetermined.

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THE INFLUENCE OF HYPERPNEA AND OF VARIATIONS OF O₂- AND CO₂-TENSION IN THE INSPIRED AIR UPON HEARING

ERNST GELLHORN AND IRWIN G. SPIESMAN

*From the Departments of Physiology and of Otolaryngology, College of Medicine,
University of Illinois, Chicago*

Received for publication March 9, 1935

It is generally known that in no other branch of physiological research is it more difficult to apply the results obtained on animals to human physiology than it is in observations concerning the brain. For this reason attempts have been made in recent times to perform experiments on anthropoids. However, even in these animals the study of sensory functions and their dependence on certain parts of the brain is extremely difficult as soon as a finer analysis is desired, because sensory disturbances become evident to the experimenter only in an indirect way through alterations in the motor behavior. For these reasons it seems desirable to study the functions of the central nervous system in the human, as far as circumstances permit.

The natural way of approach is, of course, that of a study of the functions of the sense organs under conditions which influence the excitability of the central nervous system. By making use of a spinal irradiation induced by peripheral stimuli some deeper insight into spinal physiological processes has been gained in previous experiments dealing with cutaneous sensations (Gellhorn, 1931-33). The present series of investigations concerns an alteration of the excitability of the cortex in humans and the effects brought about on various sensations, which were studied quantitatively. The factors to be investigated in this paper are variations in the O₂-CO₂-tension in the respiratory air, and thus indirectly in the blood and tissues. They were chosen not only because of the generally recognized importance of O₂ and CO₂ for the excitability of any living substance in general and of the nervous system in particular but also because of the rapidity of the gas exchange which restores normal conditions in the blood almost immediately. One of these factors (O₂-lack) has been frequently investigated for theoretical and practical reasons (aviation). However, even here a quantitative study in regard to sensations is only in the beginning. The relevant papers will be discussed later.

METHOD. The hearing experiments were conducted in a nearly sound-

proof room with a 2A Western Electric audiometer with which the threshold for hearing can be determined for eight different frequencies. The intensity is expressed in sensation units in percentages of the average hearing threshold for normal individuals. Ninety-six experiments were performed on six subjects who, after having been trained for several weeks, gave an almost absolute constancy of the hearing threshold. The procedure was as follows: The subject was connected with an audiometer and just prior to the release of the sound, a signal was given to start. Then followed sounds lasting from one to two seconds each in irregular intervals, starting with a sound twenty per cent stronger than that corresponding to the threshold and then gradually decreasing its intensity until the threshold was reached. The latter is defined by the correct response of the subject in three successive trials. The determinations were made in intervals of from 1 and 5 minutes since it had been found that readings taken in 1-minute intervals may cause fatigue particularly in experiments which extend over more than 40 to 60 minutes. The gas mixtures were prepared in several Douglas bags. The gas analysis was made with a Newcomer-Haldane apparatus.

RESULTS. *Oxygen-lack.* The first group of experiments is concerned with the influence of O_2 -lack on hearing. Thirty-three experiments were made with O_2 concentrations varying from 7.5 to 15.8 per cent. The duration of the O_2 -lack period varied between 8 and 30 minutes.

Oxygen-air mixtures of from 9 to 12 per cent O_2 showed a very marked effect. There was a gradual decrease in hearing, which began more or less rapidly, depending on the individual sensitivity of the experimental subject, the O_2 -concentration used, and the duration of the O_2 -lack period. In some cases the effects were immediately reversible after readmission of air; in others the hearing acuity remained diminished for various lengths of time.

Two experiments performed on the same experimental subject on different days are reproduced in figure 1. In both experiments the same concentration of oxygen was inhaled (10.4 per cent), but the duration of the O_2 -lack period varied considerably. With the increasing duration the effect on hearing was intensified and the recovery delayed. In contrast to these findings it must be stated that the respiration returned to normal almost immediately after the readministration of air.

A series of experiments was then conducted to determine whether the slow recovery observed after a long period of O_2 -lack could be hastened by the administration of 50 to 60 per cent O_2 . The experiment reproduced in table 1 shows that breathing of such an O_2 -rich mixture for more than 10 minutes does not appreciably alter the hearing threshold, a fact which, as will be shown later, seems to point definitely to a prolonged cellular change as a result of O_2 -lack.

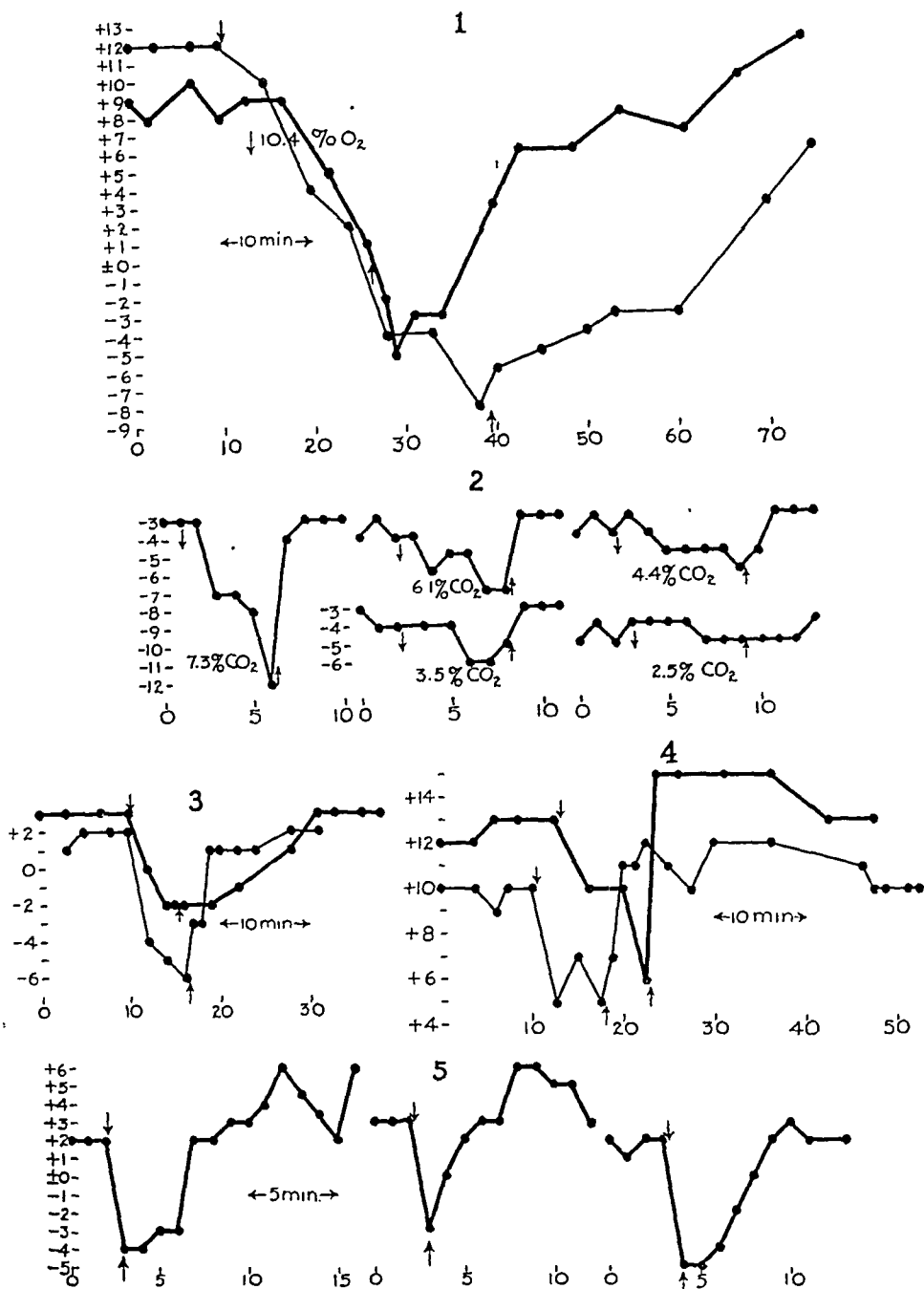


Fig. 1. In all figures: ordinate = auditory threshold in sensation units in percentage of the average human threshold for hearing. Abscissa \doteq time in minutes. Between the arrows administration of N_2 -air mixtures: In both experiments 10.4 per cent O_2 .

Fig. 2. Between the arrows CO_2 as indicated on the graph. All experiments were done on the same person.

Fig. 3. Heavy line: 5.6 per cent CO_2 . Thin line: 6.3 per cent CO_2 .

Fig. 4. Heavy line: 7.05 per cent CO_2 . Thin line: 6.9 per cent CO_2 .

Fig. 5. Three experiments with hyperpnea (between the arrows) of 2 minutes each.

Oxygen in a concentration of 12 to 15 per cent inhaled for about 10 to 20 minutes sometimes produced a slight increase in hearing during the first few minutes, followed by a gradual decrease. The effects were slight but quite distinct, in view of the great constancy of the controls obtained before and after the O₂-lack period. They were readily reversible upon readministration of air. That we are here concerned with the effect of O₂-lack and not with other factors introduced by the breathing of a gas

TABLE 1

I. SUBJ.: ST. 5/21/34. SOUND: C1024

Time	Audiometer reading	Time	Audiometer reading
A. Control period (air)		D. Inhalation of 50 per cent O ₂ for 17 minutes	
<i>minutes</i>		<i>minutes</i>	
0	+8	11	+3!!
2	+8	17	+6
3	+9		
6	+8	E. Control period (air)	
7	+9	7	+6
B. Inhalation of 10.0 per cent O ₂ for 30 minutes		12	+6
8	+6	17	+4
14	+3	74	+7
18	+3	75	+8
24	+1	77	+7
28	-2		
30	-3		
C. Control period (air) after O ₂ -lack			
2	-2		
6	-1		
11	-3		
21	-2		
29	+2		

from a Douglas bag was shown by the control experiments in which the threshold was not altered when ordinary air was inhaled.

The typical course in all experiments described so far was that during the period of O₂-lack hearing was gradually decreased and was restored by the inhalation of air with a rapidity depending on the concentration of O₂ and the duration of the O₂-lack period. It must, however, be mentioned that in some of our experiments an improvement in hearing above the ordinary threshold was noticed during the first minutes when air was again inhaled. The improvement in hearing did not amount to more than

two or three points ordinarily, but in a few experiments quite excessive improvements lasting for several minutes were observed.

Experiments with CO₂. Thirty-seven experiments with CO₂ varying from 2 to 8.4 per cent (5-22 minutes' duration of exposure) were performed. Figure 2 shows the records of five experiments performed on the same individual with varying CO₂ concentrations. It is evident that 2.5 per cent CO₂ is without effect on the hearing threshold and that the first slight effect is seen with 3.5 per cent CO₂. With increasing concentration the hearing becomes progressively less. The experiments with other subjects gave very similar results, the threshold being around 4 per cent for an experiment lasting on an average about 6 to 10 minutes. Obviously, the mechanism involved in hearing is far less sensitive to CO₂ than the respiratory mechanism, since the breathing of 2.5 per cent CO₂ shows considerable change in the pneumographic record. Figure 3 shows that in CO₂ concentrations of about 6 per cent the decrease in hearing may persist for several minutes after readmission of air but it may be said that the recovery was in all experiments considerably faster than in experiments with O₂ of about 10 per cent, and of similar duration. Figure 4 illustrates not only the decrease in hearing resulting from the inhalation of 7 per cent CO₂ but also a very considerable improvement in hearing immediately after the readministration of air. As can be seen from the graphs, this improvement lasts over a considerable length of time and is then gradually replaced by the ordinary hearing threshold.

Hyperpnea and hearing. Twenty-six experiments were performed in which the influence of hyperpnea on hearing was studied. The experimental subjects inhaled as deeply as possible for 3 to 6 minutes according to the rhythm of a metronome. In some experiments the frequency was 35 per minute, in others considerably higher, up to 90 per minute. The results were consistent throughout the whole series of experiments and showed that immediately following the period of hyperpnea hearing was greatly decreased. Readings were not taken during the period of hyperpnea because of the impossibility of concentrating and breathing violently at the same time. Figure 5 shows several typical experiments. It indicates that a decrease in hearing may last as long as 10 minutes after such a period of hyperpnea. Therefore, it is present not only during the apnea but also at a time at which breathing has become perfectly normal. Whereas the third curve in figure 5 may be taken as a representative of experiments which showed a decrease in hearing after hyperpnea and a gradual recovery afterwards to a normal hearing threshold, the other two experiments indicate that after the threshold has become normal it may be followed by a period of hyperacusis.

Most experiments were carried out with C 1024 because we found a greater constancy of the hearing threshold for this than for any other tone.

However, when tones between C 128 and C 4096 were used similar results were obtained.

DISCUSSION. Summarizing these experiments, it may be said that under conditions of O₂-lack, CO₂-excess, and CO₂-lack, hearing is gradually decreased depending on the duration of these periods and the degree of change involved. After restoration of normal conditions hearing returns to the normal threshold. In some cases prior to this a period of hyperacusis may be observed in all three conditions. The changes induced by CO₂ are more readily reversible than those with O₂-lack and hyperpnea. In experiments with O₂-lack changes in threshold (decrease in hearing) were observed as long as two hours after the experiment.

The question arises as to the nature of the decrease in hearing observed in all three groups of experiments. There is a possibility that diminished hearing is due not to a diminished excitability of the fundamental processes involved in hearing but to an inability of the experimental subject to "concentrate the attention." Unfortunately, we do not know what attention is nor what physiological processes are called into play when we direct our attention to a sensation and perception. We only know that this peculiar process makes the sensations more vivid and distinct. It is, therefore, at least theoretically possible that a decrease in "attention" gives results similar to those obtained when the excitability is decreased. We believe, however, that this interpretation of our results is ruled out for the following reasons: First, the long training of the experimental subjects which made it highly improbable that the diminution in hearing was obtained because they were unable to concentrate their attention on the experiment; second, it was found that not infrequently at the end of the O₂-lack or hyperpnea period a period of improved hearing was obtained. This certainly could not have been observed with a diminished attention; third, measurement of attention (Bourdon test), to be reported elsewhere, showed only slight disturbances in attention which never persisted after air had been readmitted.

Instead of assuming that alterations in attention are responsible for the effects on hearing, an assumption which is highly improbable because of the great constancy of the controls before and after the experiment, it seems more reasonable to interpret the variations as being due to changes in the nervous mechanism involved in the sensation itself. Such an interpretation seems still more justified since our observations on the effect of O₂-lack are paralleled by Schubert's observations (1934) on the motor effect of O₂-lack in animals. He found that under the influence of a decreased oxygen tension in the air animals showed a decreased motor excitability leading to coma. After readmission of air an increased motor excitability was observed, leading even to convulsions. In view of these facts, we believe that the effects of O₂-lack and variations in the CO₂ tension lead to alterations in the excitability of the fundamental nervous mechanism.

We may then ask: Are these changes due to alterations in the circulation, or are they of cellular origin? It is well known that the inhalation of CO₂ and hyperpnea results in changes in blood pressure. We found, however, that in experiments on healthy, young individuals changes in blood pressure during and following the exposure to O₂-lack and CO₂-excess, as well as during hyperpnea, were extremely small. The greatest changes were observed with CO₂ and amounted to about 10 mm. mercury (systolic blood pressure). The decrease in blood pressure during hyperpnea was practically negligible and not outside of the experimental error. These findings agree well with the measurements of Voit (1933) and Raab (1929). That small changes in blood pressure are not responsible for the effects on hearing is borne out by experiments in which a considerably greater increase in blood pressure was obtained after physical exercise (20–30 mm. Hg) without influencing the hearing threshold. Furthermore, it must be emphasized that not infrequently long after-effects on hearing were observed after hyperpnea and O₂-lack, although the blood pressure remained completely unchanged.

Whereas a change in blood pressure can be eliminated as a cause of the effects on hearing observed in our experiments, the vasomotor reactions due to changes in the O₂- and CO₂-tension of the blood require a more detailed consideration. Schmidt and Pierson (1934) and Schmidt (1934) have shown that these factors alter the blood supply of the medulla oblongata and of the hypothalamus. Furthermore, direct observations of the pial vessels by Cobb and Fremont-Smith (1931), Wolff and Lennox (1930) and Lennox and Gibbs (1932) lead to similar results. It must, therefore, be assumed that both O₂-lack and CO₂-excess produce vasodilatation and increased blood flow, whereas the reduction in CO₂-tension in hyperpnea is accompanied by a reduction in the blood supply of the brain. The reduction in hearing during O₂-lack occurs, of course, not because of the increased blood supply but in spite of it. Obviously the increased blood supply cannot fully compensate for the diminished O₂-tension and as a result, the O₂-supply of the brain is reduced, although to a lesser extent than was to be expected without the vasomotor adjustment. We, therefore, obtain a diminished excitability, particularly of the cortical elements involved in hearing, since they are more sensitive to O₂-lack than any other elements of the central nervous system (concerning the literature compare Gildea and Cobb, 1930).

The very marked persistence of the effects of O₂-lack on hearing is well in line with the observations of Gildea and Cobb following a temporary complete anoxia of the brain, and seems to point to the ganglion cells of the cortex as the site of the changes. This assumption is supported by histological studies of these authors, who found the most pronounced O₂-lack effects in lamina III and IV of the cortex. As far as our own

observations are concerned, the fact that the effects of O_2 -lack on hearing are independent of the frequency of the sound used seems in the light of experiments of Percy (1929) to favor such an interpretation. So do those experiments in which 50 to 60 per cent O_2 was administered after a period of O_2 -lack. The fact that even after a 5 or 10 minute period of inhalation of 50 per cent oxygen no appreciable change in threshold occurred, seems to indicate a cellular change which persists in spite of the complete oxygenation of the blood.

The decrease in hearing obtained under the influence of CO_2 -excess is in agreement with other effects of CO_2 on the central nervous system. King, Garrey and Bryan (1932) have shown that the knee jerk is decreased in the intact animal. Obviously CO_2 acts simply on the basis of its acid properties since Fröhlich and Solé (1924) have found that by perfusion of the spinal cord with acid solutions reflex activity completely disappears.

The explanation of the reduction in hearing observed after hyperpnea seems to be more complex. We must here distinguish the effects of increased alkalinity on blood vessels and on neurons. The latter seem to show increased excitability due to increased conductivity (Broemser, 1925) and a shortening of the relatively refractory period (Strughold and Jörg, 1933). This leads to increased patellar reflexes in man and animals. On the other hand, Cobb and Schmidt and their co-workers found that hyperpnea leads to a decreased blood supply of the brain through vasoconstriction, which is not compensated for by the systemic blood pressure, since it remains either unchanged or drops slightly. The effects observed in our experiments are best understood by the not unreasonable assumption that the specific effects of decreased CO_2 -tension in the blood are more than overcompensated by the vasoconstriction. We come, therefore, to the conclusion that the effects of hyperpnea on hearing are in the last analysis effects of O_2 -lack.

In this respect observations of Cobb and Fremont-Smith (1931) in man are of great importance. They showed that, whereas CO_2 -excess increases the pressure of the cerebro-spinal fluid, hyperpnea lowers it. In contrast to the quick restoration of the normal pressure level after readmission of air following the inhalation of CO_2 , it was found that the pressure remained diminished for 7 minutes and more after hyperpnea was discontinued. This seems to indicate that the vasoconstriction caused by hyperpnea is only slowly reversible and accounts for the distinct after-effects of hyperpnea on hearing. It may, therefore, well be that all the effects of hyperpnea observed in our experiments can be explained fully on the basis of the vasomotor changes. It seems, however, not wise to make too sharp a distinction between the cellular effects of O_2 -lack in our experiments with inhalation of low O_2 -mixtures and the vasomotor effects of hyperpnea,

because it is to be expected that a sufficiently prolonged hyperpnea will result in cellular changes due to sustained vasoconstriction.

Comparing the results of our work with those of other investigators, it may be stated that Lewis (1918 and 1919) and Bagby (1921) were unable to observe any changes in hearing due to O_2 -lack except immediately before collapse. The differences are probably due to the fact that these authors applied the tests in routine experiments, whereas our observations were performed on persons trained for several weeks or months prior to the main group of experiments described in this paper. Concerning CO_2 -excess or CO_2 -lack, no experimental work seems to have been reported in the literature except for some statements of Rosett (1924), who finds that during hyperpnea the sensitivity to pain and deep pressure was increased. But it is quite obvious, from his description, that this increased response on the part of the experimental subject was not due to a change in the excitability but to an alteration which concerned particularly "affective reactions" of the experimental subject. When weak stimuli were used a diminution in response (although without quantitative measurements) was observed by him.

The experiments of Brody and Dusser de Barenne (1932) on the excitability of the motor cortex show that it is difficult to obtain any effect with hyperventilation. On local application of strychnine, however, hyperventilation did produce increased excitability to electrical stimuli. Whether this effect is due to subcortical or cortical effects may be uncertain. In addition one must always take into account that the effect of hyperpnea on cortical excitability (motor or sensory) is due to the interaction of at least two factors: 1, the effect of increased OH -concentration causing increased excitability; 2, the depressing effect of O_2 -lack due to vasoconstriction accompanying hyperpnea. The final results, therefore, may vary according to the special conditions and the quantitative reactivity of the tissue to these factors.

CONCLUSIONS

If air with oxygen content of about 10 per cent or less is inhaled for 10 to 30 minutes a decrease in the hearing threshold is observed which, dependent on the duration of the experiment, the concentration of the oxygen and the sensitivity of the experimental subject, may last up to several hours. CO_2 in concentrations of 4 to 8 per cent in air of normal O_2 content also causes a diminution in hearing which, however, is more quickly reversible. After a period of voluntary hyperpnea lasting from 3 to 6 minutes a considerable decrease in hearing is observed during the period of apnea and afterwards, until gradually the original threshold is obtained. In all three groups of experiments a supernormal phase may

temporarily be observed when normal air is readmitted. The changes in hearing seem to be due to chemical cellular alterations in the central nervous system itself.

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ON THE RENAL EXCRETION OF UREA¹

R. DOMINGUEZ

From the Laboratories of Saint Luke's Hospital, Cleveland

Received for publication March 25, 1935

Of the numerous facts concerning the renal excretion of urea the following will be retained as sufficiently well established to warrant theoretical treatment:

Fact 1. The rate of excretion per unit plasma concentration increases continuously with diuresis up to a narrow range of moderate rates of urine flow. This fact was given explicitly by Austin, Stillman and Van Slyke (1921).

Fact 2. For larger diuresis the rate of urea excretion per unit plasma concentration is independent of urine flow. This fact is due to several investigators beginning with the work of Marshall and Davis (1914). (For historical development see Möller, McIntosh and Van Slyke, 1928.)

Fact 3. The rate of excretion per unit plasma concentration depends also on protein intake. This fact we owe to Addis and Drury (1923a) and especially to Jolliffe and Smith (1931a), although the fact itself can be discovered in the early observations on the dog in the paper of Austin, Stillman and Van Slyke (1921).

The rate of excretion per unit plasma concentration has been called "clearance" by Möller, McIntosh and Van Slyke (1928). If the rate of excretion is represented by y and the plasma concentration by x , the three facts mentioned before can be symbolized by

$$(y/x) = \varphi(v, d) \quad (1)$$

where φ stands for some unknown function of diuresis, v , and diet, d . Since the physiological changes in urine flow are or can be made short in duration, relative to the changes induced by diet, it will be possible to determine the variation in (y/x) with diuresis, for a given stationary or average effect of diet. Under these conditions, equation 1 becomes

$$(y/x) = f(v) \quad (2)$$

In this paper I shall try to determine the form of the function f in equation 2. Data available at present on the effect of diet are insufficient to

¹ An abstract of part of this paper was presented at the Detroit Meeting of the American Physiological Society, April 11, 1935.

determine the function φ of equation 1. However, I shall make use of the interesting fact, also brought out by Jolliffe and Smith (1931a), that the lowering in the clearance produced by a cracker meal diet is accompanied by a shift in the range of diuresis at which the clearance becomes independent of urine flow.

From facts 1 and 2 above it is to be expected that as diuresis increases the clearance would become asymptotically equal to a constant, yet none of the expressions so far proposed for it satisfy this expectation. For instance, the equation of Ambard (Ambard and Weill, 1912) can be written

$$\frac{y}{x} = ax^{\frac{1}{2}}v^{\frac{1}{2}} \quad (3)$$

that of Austin, Stillman and Van Slyke (1921),

$$\frac{y}{x} = bv^{\frac{1}{2}} \quad (4)$$

and that of Walker and Rowe (1927),

$$\frac{y}{x} = cx^{.38}, \quad (5)$$

none of which have an asymptote. All the above equations can be put in the form

$$y = px^{\alpha} v^{\beta} \quad (6)$$

but it will be enough to consider equation 4, since the computation of the constants in equation 6 (Conway and Kane, 1929), from data in the literature, yields values sufficiently close to those of the formula of Austin, Stillman and Van Slyke (1921).

The equation for the clearance at large urine flows may be written

$$y/x = A \quad (7)$$

It will be seen that the curve represented by equation 4 will not join the curve of equation 7 along a common tangent, but will intersect it at a definite angle. Once the parameters b (equation 4) and A (equation 7) have been ascertained, by the data or otherwise, the point of intersection is definitely determined. The diuresis corresponding to it has been called "augmentation limit" by Austin, Stillman and Van Slyke (1921). These authors take, therefore, the curve of equation 4 from $v = 0$ to the point of intersection with the curve of equation 7, to represent the ratio, now called clearance, at low or moderate diuresis, and the curve of equation 7 from the augmentation limit on, to represent the clearance for large urine flows.

However well justified from an empirical point of view, this procedure is theoretically unsatisfactory. It leads, moreover, to a few inconsistencies, which I shall indicate.

The first one refers to the dimensions of the constants b and A . The dimensions of A are those of flow [l^3t^{-1}], like v itself, while the dimensions of b are [l^3t^{-1}]. Now, Möller, McIntosh and Van Slyke (1928) call A the maximum clearance, and b the standard clearance, but from the dimensional standpoint it is clear that if A is a clearance, b is not. The argument that b has the dimensions of flow at $v = 1$ is not valid, because this will depend on the units in which the flow is determined, and the choice of units is evidently arbitrary.

A second inconsistency appears when the "standard clearance" is calculated in young children or in dogs, in whom the augmentation limit is less than 1 cc. per minute. For in this case, although the clearance (y/x) is smaller at diuresis below the augmentation limit than above this limit, as it should be, the standard clearance b is larger than the *maximum* clearance A . For example, in table 1 (McIntosh, Möller, and Van Slyke 1928), the maximum clearance of the first, second, third, fifth and sixth child is, respectively, 19.8, 27.9, 18.3, 24.2 and 38.9, while the standard clearance is, in the same order, 45.0, 35.9, 38.7, 26.9 and 40.8. Similarly, in Jolliffe and Smith's paper (1931b) the maximum clearance of the dog is, on an average, 58.0, and the standard clearance 92.8, both calculated per unit of body surface. The situation would not be improved by saying that the standard clearance is the clearance referred to the diuresis $v = 1$, because if the augmentation limit is less than 1, let us say, 0.4 cc. per minute, as in the dog, equation 4 would not apply at diuresis greater than 0.4 cc. per minute, and consequently the ratio (y/x) calculated from equation 4 at $v = 1$ would be meaningless. As a matter of fact, with such an interpretation, the two numbers given by Jolliffe and Smith (1931b) would mean that the average dog has two clearances at $v = 1$, a maximum clearance of 58.0 and a standard clearance of 92.8.

In order to avoid the foregoing difficulties I have proceeded in a different way.

Equation 7 may be written

$$y = Ax \tag{8}$$

This means that the excretion of urea at large diuresis is of the same form as that of exogenous creatinine and xylose at any diuresis (Dominguez and Pomerene, 1934a, 1934b). Let us assume that at low urine flow urea is absorbed in the kidney itself. Then the rate of excretion y , as determined in the ordinary way, may be regarded as the resultant of two operations: first, the rate s at which urea is poured into the lumen of the

tubules, and second, the rate r at which urea is absorbed from the lumen of the tubules. In symbols

$$y = s - r \quad (9)$$

at any diuresis.

In order to determine y it is necessary to make some assumptions concerning the nature of the two rates s and r . The two assumptions are:

1. At any diuresis, the rate at which urea is removed from the blood is proportional to the concentration in the plasma, that is,

$$s = A x \quad (10)$$

2. The rate at which urea is absorbed varies with diuresis in such a way that the instantaneous change in the rate of absorption r with respect to diuresis v , is proportional to the rate of absorption r . In symbols,

$$\frac{dr}{dv} = -kr \quad (11)$$

where k is a constant. The minus sign indicates that the rate of absorption decreases as diuresis increases.

The integration of equation 11 gives

$$r = c e^{-kv} \quad (12)$$

Substituting s and r from equations 10 and 12 in equation 9, we get

$$y = A x - c e^{-kv} \quad (13)$$

and since at $v = 0$, $y = 0$, it follows that

$$c = A x \quad (14)$$

Therefore,

$$y = A x (1 - e^{-kv}) \quad (15)$$

or in terms of the ratio (y/x) ,

$$(y/x) = A (1 - e^{-kv}) \quad (16)$$

at any diuresis.

From equation 10, A has the dimensions of flow, and from equation 11, k has the dimensions of the reciprocal of flow. Therefore, the dimensions of equation 16 are satisfied at any diuresis. It should be noted that the product (Ak) has the dimensions of a pure number, a result which will be used later.

Equation 16 shows that as diuresis increases, the rate of excretion per

unit plasma concentration (clearance) increases asymptotically to a mean value A , provided the effect of diet is stationary. The notion of an augmentation limit drops out. In practice, however, a region of v exists in which the clearance is practically indistinguishable from its asymptotic value A . The left boundary of this region (in an ordinary Cartesian diagram) may still be called "augmentation limit," but the mean value A will be designated by "asymptotic" or "stationary clearance," in preference to maximum clearance, because the expression for the clearance (equation 16) has no maximum.

TABLE 1

Urea clearance C in man. Data by Möller, McIntosh and Van Slyke (1928)

v	C	n	NUMBER OF SUBJECTS	S	$k \cdot \log e$	Δ_1	b	Δ_2
0.308	22.02	4	4	1.765	0.527	-1.90	39.68	-5.27
0.518	38.94	11	8	1.704	0.673	+3.55	54.10	+3.55
0.683	47.16	17	11	1.808	0.702	+4.77	57.06	+6.52
0.867	48.33	7	5	1.718	0.578	-0.23	51.90	+2.55
1.057	48.63	9	6	1.757	0.480	-4.89	47.30	-1.92
1.288	54.58	6	3	1.735	0.500	-3.49	48.09	-1.22
1.513	62.73	3	3	1.687	0.631	+1.40	51.00	+2.25
1.675	61.30	6	4	1.660	0.526	-1.84	47.36	-2.34
1.815	68.35	2	2	1.675	0.828	+3.93	50.73	+2.11
2.047	63.67	3	3	1.627	0.493	-2.39	44.50	-6.68
2.420	65.77	3	3	1.617	0.482	-2.07		(-10.72)
≥ 2.60	70.57	33	6	1.728				

v = mean diuresis, cubic centimeters per minute.

C = mean clearance, cubic centimeters per minute.

n = number of observations.

S = body surface, weighted mean, square meters.

k = from equation 16 with $A = 70.57$.

Δ_1 = difference between observed clearance and clearance calculated from equation 17.

b = from equation 4.

Δ_2 = difference between observed clearance and points on the curve $C = 49.17\sqrt{v}$.

The assumptions leading to equation 16 have been made very broad in order to embrace several possible schemes of renal physiology, in so far as the function of the structural units of the kidney is concerned. The identification of the rates s and r of equation 9 with the scheme of glomerular filtration and tubular absorption is not possible at the present time.

Urea clearance in man. The published data of Möller, McIntosh and Van Slyke (1928) were grouped as shown in table 1 in order to test the applicability of equation 16 to the mean clearance in man. The data

are not as numerous as could be desired, but they have the advantage of giving information on the size of the subject. An interval of diuresis of 0.20 cc. per minute was taken as unit of grouping, closing the interval on the left side, thus $0.20 \leq v < 0.40$. From a diuresis of 2.60 up to 16.25 cc. per minute the observations yield a mean clearance equal to 70.57, which was used as the value of A . The numerical work is given in table 1. The mean value of k is 1.344. Equation 16 with the values of A and k for man becomes

$$C = 70.57 (1 - e^{-1.344v}) \quad (17)$$

With the help of this equation the theoretical values of the clearances were then calculated at each diuresis and the differences between the observed and calculated clearance (residuals Δ_1) computed. The variance (sum of the squares of the residuals divided by the number of points minus one) is 10.5898.

For the purpose of comparison, the constant b of equation 4 was also calculated from the first 10 means, as shown in table 1, and the residuals Δ_2 were determined as before. The variance was found to be 17.1306, showing that the equation of Austin, Stillman and Van Slyke (1921) fits the mean data of Möller, McIntosh and Van Slyke (1928) at low and moderate diuresis less well than equation 16 at any diuresis (see fig. 1). There is no correlation between the deviations from the mean body surface and the residuals corresponding to equation 17, so that the large irregularities in the mean clearances are probably all due to the small number of observations.

In view of the previous analysis of Conway and Kane (1929) on earlier observations on urea excretion, the fit of equation 16 to the data of Möller, McIntosh and Van Slyke (1928) was not anticipated, because this equation does not fit the means of Conway and Kane as well as equation 4. The problem with which I am concerned here is not one of fitting a curve to a set of points. If the stationary value of the clearance at large diuresis is considered as established, then a relation of the form of equation 6 cannot be accepted, regardless of whether it fits a group of observations in an arbitrarily restricted range of diuresis. Besides, the data collected by Conway and Kane are not entirely homogeneous, since their figures include data on fasting subjects and on others "where there was apparently much variety in the water and nitrogen intake." Under these circumstances the danger of introducing systematic deviations could hardly be avoided.

Urea clearance in the dog. For the clearance of the dog I have made use of a few published data, as will be indicated below, and especially of the numerous observations of Dr. H. Goldblatt, of the Department of Pathology, Medical School, Western Reserve University, which he has very kindly put at my disposal. Altogether Doctor Goldblatt's data consist of

108 clearances at diuresis larger than 0.5 cc. per minute and 364 clearances below 0.5 cc. per minute, not including data in which the body surface of the animal was not known. These observations were carefully examined, all the ratios recalculated, and the body surfaces computed by formula 1 of Cowgill and Drabkin (1927). The mean clearance of the first series of 15 dogs, at diuresis larger than 0.5 cc. per minute, is 38.1, with a mean body surface = 0.691 square meter. In the second series of 9 dogs, the mean clearance = 31.0, and mean body surface = 0.629.

The mean clearance and mean body surface of each dog were next determined in order to estimate the correlation between the two measurements.

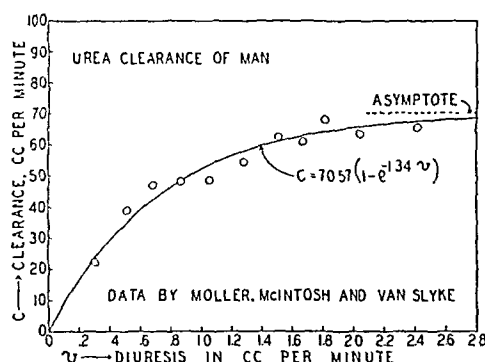


Fig. 1

Fig. 1. Urea clearance of man. The circles represent the mean clearances of Möller, McIntosh and Van Slyke (1928) after the grouping indicated in table 1. The smooth curve has been calculated from the equation $C = 70.57(1 - e^{-1.34v})$. Part of the asymptote $A = 70.57$, stationary clearance at large urine flow, has been indicated by a broken line.

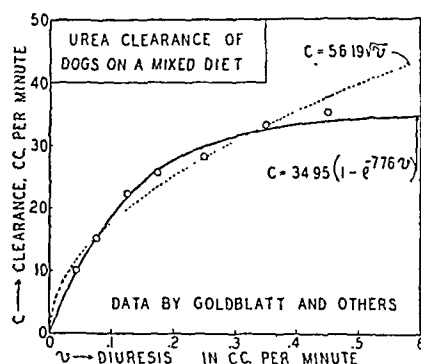


Fig. 2

Fig. 2. Urea clearance of dogs maintained on a mixed diet. Data from the sources indicated in the text. Each circle represents the mean clearance of a number of observations (see table 2) in a given range of diuresis. The solid curve corresponds to equation 16 with $A = 34.95$ and $k = 7.76$. The asymptote of the curve has not been drawn in order to avoid confusion. The broken line corresponds to equation 4, with b ("standard clearance" of Möller, McIntosh and Van Slyke) equal to 56.19.

Fifteen pairs of observations were obtained from the first series and nine from the second. Five more pairs were added from the data of Jolliffe and Smith (1931b) and Jolliffe, Shannon and Smith (1932), after grouping the observations per dog.

The correlation between the clearance at $v \geq 0.5$ cc. per minute and the body surface of the dog is significant (correlation = 0.5608, number of pairs of observation = 29, see Fisher, 1928, table V. A). The two regression equations are

$$S = 0.506 + 0.00455 A \quad (18)$$

$$A = -9.94 + 69.07 S \quad (19)$$

the range of S being between 0.50 and 0.82 m².

For the part of the curve between $v = 0$ and $v = 0.5$ cc. per minute, I have put the (unpublished) data of Goldblatt together with those of Ralli, Brown and Pariente (1931), and of Jolliffe and Smith (1931b), after a preliminary rough grouping had shown that the four series follow the same trend. The whole material consists of 432 observations as follows:

	Number of dogs	Mean body surface	Number of observations
Goldblatt's 1. series.....	17	0.673	150
Goldblatt's 2. series.....	16	0.612	214
Jolliffe and Smith.....	7	0.700	19
Ralli, <i>et al.</i>	9	0.563	49
	49	0.637	432

If the data are grouped by intervals of 0.1 square meter of body surface and also by intervals of 0.05 cc. per minute of urine flow, the number of observations in most of the groups is found very small. Even if the interval of diuresis is increased to 0.1, between 0.2 and 0.5 cc. per minute, the results are not much better. It is impossible, therefore, to get any idea of the correlation between body size and clearance at low diuresis, but it is possible to derive information on the mean clearance per unit of diuresis for the whole group of dogs. The mean clearances were grouped, therefore, first, by taking the mean of the means per unit of body surface, and second, by multiplying by the number of observations in each compartment (weighted mean). To each mean clearance of the first grouping there corresponds an associated mean body surface and to each mean clearance of the second grouping a weighted mean body surface.

The numerical work is presented in tables 2 and 3. The intervals of diuresis are equal to 0.05 cc. per minute from zero to 0.2, and 0.10 from then on. The value of v in the tables represents the middle of the interval, except the first one. In this interval, v is actually the mean diuresis of the group, since there are no observations below 0.025 cc. per minute. In the other intervals the means are so close to the middle of the intervals that it is immaterial which ones are used.

The value of A for the first grouping was calculated from equation 19, taking $S = 0.650$. The mean value of k is 7.76. The theoretical clearances were then calculated from the equation

$$(y/x) = 34.95 (1 - e^{-7.76v}) \quad (20)$$

and the residuals Δ_1 computed, as shown in table 2. The variance is 0.9889. For comparison the constant b of equation 4, and the corresponding residuals were also computed (see table 2). The variance in this case is 2.5693, indicating a less good fit than equation 16. Analogous calculations with the data of the second grouping yield the equation

$$(y/x) = 36.00 (1 - e^{-7.28v}) \quad (21)$$

with residuals as shown in table 3. The variance is 1.0535, not much different than that from equation 20, while the variance obtained from equation 4, "standard clearance" b , is 3.7519. Equation 16 is, therefore, verified by independent observations on dogs maintained on a mixed diet, for a wide range of diuresis (see fig. 2).

TABLE 2
Urea clearance in the dog (mixed diet). First grouping

v	C	S	k.log e	Δ_1	b	Δ_2
0.042	10.00	0.606	3.4848	+0.28	48.80	-1.52
0.075	15.08	0.650	3.2703	+0.01	55.06	-0.31
0.125	22.28	0.650	3.5255	+0.58	63.02	+2.41
0.175	25.66	0.650	3.2882	-0.46	61.34	+2.15
0.25	28.17	0.650	2.8488	-1.75	56.34	+0.07
0.35	33.31	0.650	3.7966	+0.67	56.30	+0.07
0.45	35.22	0.651		+1.34	52.50	

k = from equation 16 with $A = 34.95$.

Δ_1 = difference between observed clearance and clearance calculated from equation 19.

Δ_2 = difference between observed clearance and points on the curve $C = 56.19\sqrt{v}$. Other symbols as in table 1.

TABLE 3
Urea clearance in the dog (mixed diet). Second grouping

v	C	S	n	k.log e	Δ_1	b	Δ_2
0.042	8.44	0.528	16	2.7618	-1.05	41.18	-3.03
0.075	15.65	0.607	112	3.3029	+0.50	57.14	+0.32
0.125	22.06	0.630	95	3.2965	+0.54	62.40	+2.27
0.175	24.57	0.638	75	2.8472	-1.37	58.73	+1.15
0.25	29.10	0.679	75	2.8695	-1.07	58.20	+1.11
0.35	34.45	0.686	39	3.9015	+1.26	58.23	+1.33
0.45	35.60	0.661	20	4.3437	+0.26		

C = clearance, cubic centimeters per minute, weighted mean.

S = body surface, square meter, weighted mean.

k = from equation 16 with $A = 36.00$.

Δ_1 = difference between observed clearance and clearance calculated from equation 20.

Δ_2 = difference between observed clearance and points on the curve $C = 55.98\sqrt{v}$. Other symbols as in table 1.

The published data on a meat diet and in the fasting state are insufficient to establish the applicability of equation 16 to the excretion of urea under those circumstances. There is, however, no *a priori* reason why it should not apply. On the assumption that it does, the equation should be re-

garded as representing a two-parameter family of curves, within the region of existence of the clearance. But the observations of McIntosh, Möller and Van Slyke (1928) on the clearance of the child (effect of growth) and those of Jolliffe and Smith (1931a) on the clearance of the dog (effect of diet) indicate that a change in the value of the stationary clearance A is accompanied by a shift in the augmentation limit. The two parameters A and k are, therefore, not free, but bound to each other in some way that limits their simultaneous variation in the sense observed by the above authors. It is possible to arrive at some such relationship by considering the limiting concentration ratio of urea.

The limiting value (Ak). If the concentration of urea in the urine is designated by u , in the same units as the concentration in the plasma, x , equation 16 can be written

$$\frac{u \cdot v}{x} = A(1 - e^{-kv}) \quad (22)$$

Collecting the v terms on the right-hand side of the equation we get

$$\frac{u}{x} = \frac{A(1 - e^{-kv})}{v} \quad (23)$$

The limit of the expression on the right-hand side as v approaches zero is equal to Ak , therefore,

$$\lim \frac{u}{x} = Ak \quad (24)$$

This means that the concentration of urea in the urine per unit plasma concentration approaches a limit as diuresis diminishes. It should be noted that the dimensions of equation 24 are satisfied.

The other equations proposed for the excretion of urea do not lead to any limiting value. For instance, the ratio $\left(\frac{u}{x}\right)$ from equation 4,

$$\frac{u}{x} = \frac{b}{\sqrt{v}}$$

would grow without limit as v diminishes. The limit of $\left(\frac{u}{x}\right)$ should not be confused with the "concentration limite" of Ambard and Papin (1909), later called "concentration maxime" by Ambard and Weill (1912). The latter refers to the concentration in the urine, u , but, from equation 23, no limit exists for u independently of x .

In the ideal limit, the value (Ak) appears as a state of equilibrium between the plasma and the urine, and such equilibrium may be expected to remain undisturbed by purely physiological variations in the activity of

the kidney. In particular, if the effects of diet belong in this category and we use the subscript d to indicate a given diet, we would be led to write

$$A_d \cdot k_d = m, \quad (25)$$

m being a constant independent of diet, as the simplest inverse relation between A and k . This hypothesis would immediately account for the shift in the augmentation limit with changes in A . Because, by differentiating equation 16 with respect to v , and writing C for (y/x) , thus

$$\frac{dC}{dv} = (Ak)e^{-kv}, \quad (26)$$

we see that the slope of the curve will become practically zero for larger diuresis when k is small than when k is large. In other words, the range of asymptotic approach (augmentation limit) will occur at larger diuresis when A is large, as on a meat diet, than when A is small, as in fasting. And this, it will be remembered, is the fact observed by Jolliffe and Smith (1931a). A similar reasoning applies to the observed change in clearance and augmentation limit in the child. Unfortunately the data bearing on both these points are too scanty for statistical treatment, so that equation 25, however suggestive, remains hypothetical.

It should be kept in mind that if the existence of a relation between A and k is imposed by empirical facts not introduced in the development of equation 16, the form of this relation does not have to be necessarily that of equation 25. Should experiments designed for the purpose indicate a different relation, the fact would not in itself invalidate equation 16.

The value (Ak) can be considered from another point of view. Since the limit of the concentration ratio (u/x) is in some regards a measure of the power of the kidney to concentrate urea, it seems as if a small carnivorous animal would be at a disadvantage if his kidneys could not concentrate more than those of an omnivorous or herbivorous animal. The only data available on this point are those on man and on the dog. From equation 17, Ak is equal to 95 for man, and from equation 20, Ak is equal to 271 for the dog. In other words, the kidney of the dog has a concentrating power about three times as large as that of man.

The same line of thought leads to a suggestive reason for the increment in the clearance with an increment in the protein intake.

In a previous paper (Dominguez and Pomerene, 1934a), it was shown that, with a constant A , ingested creatinine disappears from the blood exponentially with the time, after the absorption by the bowel has practically ceased. From this fact it was proved (Dominguez, 1934) that the constant of elimination α is given by

$$\alpha = \frac{A}{V} \quad (27)$$

where V is the volume of distribution of creatinine in the body at the same concentration as the plasma. Since at large diuresis urea is excreted according to the same law as creatinine, it is clear that, under the same circumstances as before, equation 27 should also hold for urea. Because urea is more diffusible than creatinine, it is not likely that the volume of distribution of the former should be smaller than that of the latter. On the other hand the clearance A of urea is known to be smaller than that of creatinine. It follows from equation 27 that the constant of elimination of urea must be smaller than that of creatinine.

From preliminary experiments (unpublished data; Dominguez, Goldblatt and Pomerene), the value of α on a dog weighing 20 kgm. is, for creatinine, approximately 0.18 when the time is measured in hours. This means that every 3.85 hours the concentration in the plasma is one-half of its value at the beginning of the period. Now, the value of A for creatinine in the same dog is about 49 cc. per minute, and its value for urea, at large diuresis, is approximately 32. Therefore, on the assumption that the volumes of distribution are the same, the constant of elimination of urea should be, in this dog, equal to 0.118, or smaller, if its corresponding V is larger than that of creatinine. Consequently, the half period of the fall in urea concentration is 5.87 hours. Or, in different words, at the end of 24 hours the urea concentration in the plasma would be about 5.9 per cent of its initial value. If the ingestion of urea is repeated every 24 hours, it will be apparent that, unless the constant of elimination increases in value, the dog would slowly but surely enter into a state of urea retention. If instead of the ingestion of urea we imagine that the urea to be eliminated is derived from protein intake, we know that in a relatively short time a state of equilibrium is reached, the so-called nitrogen equilibrium. This can be accomplished only by increasing the value of the constant of elimination α . From equation 27, α can increase either by diminishing V or by increasing A , and since V (which is proportional to the body weight) does not diminish, and, in fact, may increase by the process of eating, it appears that the only way protein intake can speed up the elimination of urea is by increasing the value of the clearance A .

It should be emphasized that in the above reasoning the dog has been placed in a favorable position, because I have assumed nitrogen equilibrium, a diuresis not less than 0.4 cc. per minute, and besides I have neglected *all* the urea formed during absorption. Moreover, a value of $A = 32$ cc. per minute corresponds to the mixed diet level. Had I assumed a fasting level of 23 cc. per minute for A , the constant of elimination would have been necessarily smaller and the resulting plasma concentration at the end of 24 hours larger than 6 per cent of its initial value.

From the foregoing considerations the increment in the clearance with protein digestion appears as a nice self-adjusting device, whereby the

time of the elimination of urea is shortened and an undesirable retention of waste products prevented. In fact the very existence of a nitrogen equilibrium rests on this phenomenon.

That urea is not the cause or its removal the purpose of the increase in the clearance is amply demonstrated by the failure of urea to raise its own clearance even at very large plasma concentrations (Drury, 1923). For this reason administered urea can be used in the study of urea excretion. But all researches on nitrogen metabolism in which a constant rate of excretion per unit plasma concentration has been assumed, will require revision.

The efficiency of urea excretion. From the standpoint of urea excretion the efficiency of the kidney may be defined, in analogy to mechanical efficiency, as the ratio of the urea supplied the kidney in a given time to the urea put out by the kidney in the same time. The quantity supplied is equal to the flow in the renal arteries multiplied by the concentration of urea in the arterial blood. The quantity removed from the body by the kidneys is equal to the flow of urine multiplied by the concentration of urea in the urine. If we designate the efficiency E and the arterial blood flow by B , we have by definition

$$E = \frac{v \cdot u}{B \cdot x} \quad (28)$$

In this equation equi-dimensional quantities are supposed to be measured in the same units, and since both numerator and denominator have the same dimensions the efficiency is a pure number (see however Möller, McIntosh and Van Slyke, 1928, where the clearance itself is called efficiency).

Substituting in equation 28 the value of $v \cdot u = y$ from equation 15, we get

$$E = \frac{A(1 - e^{-kv})}{B} \quad (29)$$

For large diuresis the efficiency becomes simply

$$E = \frac{A}{B} \quad (30)$$

Since A is here a mean value, it would be enough to postulate a mean blood flow through the kidneys in order to obtain a mean efficiency. In the case of the dog, I have taken the mean of the mean figures for blood flow through one kidney which have been conveniently tabulated by Van Slyke, *et al.* (1934) from many different sources. The mean is 120.7, or, if two very small values are omitted, 131.2 cc. per minute. Introducing in equation 29 the values of A and k from equation 20 we get an efficiency

varying from 0 to 14.5 per cent (or 13.3 per cent if the larger mean blood flow is taken), as diuresis increases from 0 up to any value beyond the augmentation limit. This figure means little by itself because the value of A depends on the diet, and little is known of the effect of diet on the blood flow through the kidney. Whatever it might be, a blood flow of 2 cc. per minute per gram of kidney will represent a flow of 600 cc. per minute through the two kidneys of an average adult man, and this flow together with a clearance of 70.6 cc. per minute gives an efficiency $E = 11.8$ per cent for diuresis above the augmentation limit. Or, if the figures of Burton-Opitz (quoted by Wiggers, 1923) are applied to man, namely, 150 cc. per 100 grams of kidney, we get an efficiency of 15.7 per cent.

The urea clearance of the rabbit, from the observations of Addis and Drury (1923b) on 18 rabbits, is about 4.65 cc. per minute, while it is 6.27 in the experiments of Drury (1923) on five rabbits weighing from 2.2 to 2.5 kilos. On the other hand, the blood flow through the kidneys is on an average 2 cc. per minute per gram of kidney (Kay and Sheehan, 1933), and since, according to the same authors, the mean weight of the two kidneys is 17 grams in a group of animals with a mean body weight of 2.53 kilos, the efficiency of urea excretion in the rabbit is, on an average, equal to 13.7 per cent. The figures of Drury (1923) would yield an efficiency of 18.4 per cent if the mean blood flow of 34 cc. per minute were assumed, but it is enough to take a blood flow of 2.7 cc. per minute to bring the efficiency in this case to the same level of 13.7 per cent. From table 12 of Kay and Sheehan's paper (1933), it appears that a blood flow between 1 and 3 cc. per minute per gram of kidney was recorded 127 times in a total of 200 observations, so that a flow of 2.7 cc. per minute is not excessive.

The efficiency is, therefore, of the same order of magnitude in the dog, the rabbit and man.

A quantity similar to the efficiency E (in practice if not in principle) has been recently used by Van Slyke *et al.* (1934) to estimate the blood flow through the kidneys. The equation used by these authors for the blood flow B' is, in the symbols of this paper,

$$B' = \frac{y}{x_a - x_v} \quad (31)$$

where the subscripts a and v refer to concentration of urea x in the artery and vein respectively. Dividing numerator and denominator on the right-hand side of equation 31 by x_a , we obtain

$$B' = \frac{A}{E'} \quad (32)$$

where $E' = (x_a - x_v)/x_a$ is the extraction ratio of Van Slyke. (The name originates with Dunn, Kay and Sheehan, 1931.) The formal analogy of equation 32 to equation 30 is evident.

By substituting the mean value of A and the mean value of E' from the data of Van Slyke a value is obtained for B' which agrees well with direct measurements of flow through the renal artery. But if this result applies to the mean values, it does not follow that the same holds for any estimated value of blood flow. Since the blood flow was not measured, it is clear from equation 32 that the values of B' will vary with variations in A and E' , and it would be meaningless to say that variations in A are produced by variations in B' . Therefore, the proposition that spontaneous variations in the urea clearance parallel variations in renal blood flow, cannot be proved from the data of Van Slyke, *et al.*

SUMMARY AND CONCLUSIONS

By assuming that at low diuresis the diminution in the urea clearance is due to absorption of urea in the kidney itself, and by giving to this assumption a mathematical expression, it is possible to throw the relation between clearance, C , and diuresis, v , in the form

$$C = A (1 - e^{-kv})$$

where A is the mean asymptotic value of the clearance as v increases, k a positive constant, and e the base of natural logarithms.

It is shown that, both in man and in the dog, *under ordinary diets*, the means of a large series of observations at low urine flow are more faithfully represented by the above equation than by the equation proposed by Austin, Stillman and Van Slyke. Since in the above expression the clearance becomes asymptotically equal to a constant, the equation represents the change in the mean clearance with diuresis, at any urine flow.

A consequence of the equation is the existence of a limiting concentration ratio for urea, the limit being equal to A/k as diuresis approaches zero. Since the limiting concentration ratio is a measure of the concentrating power of the kidney, it is pointed out that this limit is considerably larger in a carnivorous animal like the dog, than in an omnivorous animal like man.

Inasmuch as a change in the mean value A by diet (in the dog) or by growth (in the child) is accompanied by a shift in the range of asymptotic approach to A , it is demonstrated that a shift of this kind can be produced by assuming that the limiting concentration ratio is a constant independent of diet (or of growth). The quantitative verification of this assumption, however, has not yet been given.

A deep physiological significance is attached to the increase in the clearance A with protein intake. From previous considerations on the

simple case of creatinine excretion after absorption has practically ended, it is shown that a retention of urea would inevitably follow the ingestion of protein, unless protein intake raises in some way the clearance of urea. The existence of this phenomenon is essential for the establishment of a nitrogen equilibrium.

By defining the efficiency of urea excretion by the kidney as the ratio of the urea supplied the kidney in a given time to the urea put out by the kidney in the same time, it is possible to state that the efficiency equals the ratio of the mean clearance to the mean renal blood flow. The efficiency of urea excretion, computed from a mean clearance and a mean renal blood flow from independent sources, shows that the efficiency is of the same order of magnitude in man, dog and rabbit.

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THE EQUILIBRIUM TIME OF THE GASEOUS NITROGEN IN THE DOG'S BODY FOLLOWING CHANGES OF NITROGEN TENSION IN THE LUNGS¹

LOUIS A. SHAW, ALBERT R. BEHNKE,² ANNE C. MESSER, ROBERT M. THOMSON AND E. PREBLE MOTLEY

From the Department of Physiology, Harvard School of Public Health, Boston, Mass.

Received for publication March 13, 1935

Direct measurements have not been made in man or in animals either of the gaseous nitrogen content of the whole body or of the time required to establish equilibrium between the partial pressure of nitrogen in the lungs and in the tissues when changes in barometric pressure occur.

It is generally held that the gaseous nitrogen is dissolved in the fat and the water of the body. Vernon (1907) and Campbell and Hill (1933) have shown that nitrogen is five times as soluble in fat as in water. If the body is 15 per cent of fat by weight, then the nitrogen is about equally divided between the fat and the water.

The rate of saturation of the body with excess nitrogen when the partial pressure of nitrogen in the lungs is raised, or the rate of desaturation when the partial pressure is lowered, depends essentially on the blood flow through the lungs and the tissues and hence may be considered a function of cardiac minute volume. Zuntz (1897) assumed that when the partial pressure of the nitrogen in the body is in excess of that in the lungs, a constant percentage of the remaining excess nitrogen passes into the blood per unit of time and is eliminated through the lungs. This assumption has been represented graphically by Boycott, Damant and Haldane (1908) for any tissue or part of the body through which the blood flow is uniform. Of particular importance is the determination of the rate at which the most slowly saturating tissues come into equilibrium with changes in the nitrogen tension in the lungs. From data obtained by experiments on goats subjected to high air pressures, and from men working under excess pressure, Boycott, Damant and Haldane (1908) concluded that about 5.5 hours were necessary for 98 per cent saturation or desaturation of those tissues having the slowest rate of saturation with nitrogen.

In man, measurements of nitrogen elimination from the body when pure oxygen is inhaled were made by Bornstein (1913) for periods of approxi-

¹ This research was aided by the Miriam Smith Rand Fund.

² Member of the United States Naval Medical Corps.

mately 7 minutes. Campbell and Hill (1931) using a modification of Bornstein's method found that approximately 200 to 300 cc. of nitrogen, or approximately one-third of the total content of the body, were eliminated during the first 9 minutes. Campbell and Hill (1933) measured the nitrogen absorbed by the brain, liver and bone marrow of goats and concluded that only 50 per cent saturation was attained in these tissues after 3 to 5 hours' exposure to excess pressures.

Valuable as these studies are for individual tissues they nevertheless do not show directly the manner in which nitrogen is absorbed or eliminated by the intact animal. In the experiments presented in this paper measurements were made on dogs, showing the rate at which nitrogen equilibrium is attained in the living, intact body when exposed to varying air pressures for varying periods of time.

METHOD. The gaseous nitrogen in the body was removed by the inhalation of pure oxygen. Since the partial pressure of the nitrogen dissolved in the body is the same as that in the alveolar air, the substitution of oxygen in the lungs for the nitrogen normally present will, if time be permitted, result in the complete elimination of all the nitrogen held by the tissues. Dogs which had been deprived of food for 40 hours were anesthetized with Dial,³ 0.6 cc. per kgm. of body weight, injected intraperitoneally. Repeated experiments were frequently performed on the same dog.

The anesthetized dog was placed in a metal box having a lid which fitted into a mercury seal. The gas in the system was kept in constant circulation by means of a gas-tight blower, which was immersed in a bath of heavy oil to ensure further against leakage of air into or gas out of the blower. Upon leaving the box the gas passed through a can containing soda-lime for the absorption of carbon dioxide. The spirometer served as a reservoir from which oxygen was supplied to meet the metabolic requirements. Before returning to the box, the circulating gas passed through a coil immersed in ice water in order to condense out the excess moisture and to prevent an excessive rise in temperature.

After the dog had been inclosed in the box, the entire apparatus was rinsed out with oxygen. This process required 7 minutes. During this time the nitrogen which was eliminated from the body was lost. From estimations, based upon the subsequent nitrogen elimination, the fraction lost in this manner had a constant value of 30 ± 5 per cent of the total nitrogen content of the body. A check on this value was obtained from a post-mortem analysis of the fat and water content of dog D. The gaseous nitrogen content of the body was then equal to the weight of the fat and water multiplied by the solubility coefficient of nitrogen. From these data it was estimated that the nitrogen lost was 32.3 per cent of the total.

³ The authors wish to thank Dr. Charles C. Haskell of the Ciba Company, New York City, for his kindness in furnishing the dial-urethane solution used as the anesthetic in these experiments.

The volume of nitrogen in the apparatus at any given time was determined by multiplying the percentage of nitrogen by the total volume of gas in the apparatus, allowance being made for the gas displaced by the dog's body. The volume was corrected for water vapor tension and then reduced to standard conditions of temperature and pressure. Changes in volume could be read from the spirometer scale with an accuracy of ± 10 cc. The nitrogen concentration was determined by the manometric method of Van Slyke and Sendroy (1932). By means of check analyses the analytical error was not allowed to exceed ± 0.003 per cent. In a system containing 100 liters the analytical error was therefore ± 3 cc. of nitrogen.

Four control experiments made without a dog in the apparatus for periods of 5 hours gave very satisfactory results. In every respect the regular experimental procedure was followed. Oxygen was admitted during the experiment and the necessary corrections made for the nitrogen added with the oxygen, and for the nitrogen entering through the water seal of the spirometer. In only 1 analysis out of 11 did the observed values deviate by more than 3 cc. from the calculated values.

The concentration of nitrogen in the apparatus at the termination of an experiment was less than 1 per cent. The partial pressure of nitrogen in the body under normal atmospheric conditions is about 573 mm. Under the conditions of our experiments the partial pressure of the nitrogen in the body is reduced to 7.6 mm. or less, instead of 0 mm., so that the volume of nitrogen eliminated is about 1.3 per cent less than the true value for complete desaturation. No correction has been applied since the error involved is insignificant.

EXPERIMENTAL RESULTS. Table 1 shows the nitrogen eliminated from 7 different dogs which were in nitrogen equilibrium with air at atmospheric pressure. On 3 dogs the experiment was repeated on different days. The volume of nitrogen collected when reduced to cubic centimeters per kilogram of body weight is very constant with the exception of dog A which gave up 8.4 cc. of nitrogen per kilogram of body weight, and dog G which gave up 14.0 cc. per kilogram. The former was exceptionally thin and the latter was an old dog and moderately fat. These extreme values, therefore, may be correlated with differences in the fatty content of the tissues, since nitrogen is five times as soluble in fat as in water. Although the volume of nitrogen collected from these dogs is less than the total nitrogen contained in the body, owing to the nitrogen lost during the first 7 minutes of rinsing with oxygen, we may assume that the nitrogen collected bears approximately the same ratio to the total nitrogen in the body in each case.

Figure 1 shows typical desaturation curves of 4 dogs. Similar curves were constructed for each experiment, showing the per cent desaturation correlated with time. These data are given in the last two columns of table 1.

Whether or not the desaturation time remains constant for all pressures must depend upon the capacity of the blood to carry its increased load of nitrogen. Experimental data relative to this question are afforded by curves representing the nitrogen elimination of dog D following equilibrium established at 1, 3 and 4 atmospheres of pressure. It was then compressed for 4 hours, a period of time which was 1 hour in excess of the saturation time for this dog (see D experiments, fig. 1 and table 1), assuming that saturation time equals desaturation time, as will later be shown to be the case. At the termination of the compression period the dog was decompressed in 10 seconds and immediately placed in oxygen and desaturated of its nitrogen by the method described. If the volume of nitrogen

TABLE 1

*Desaturation from 1 atmosphere of air. The nitrogen eliminated during the first 7 minutes of oxygen inhalation was not collected**

DATE	EXPERIMENT	WEIGHT	TYPE	TOTAL NITROGEN	NITROGEN PER KILOGRAM OF BODY WEIGHT	DESATURATION TIME	
						95 per cent	100 per cent
		<i>kgm.</i>		<i>cc.</i>	<i>cc.</i>	<i>min.</i>	<i>min.</i>
Nov. 13	A	8.8	Very thin	74	8.4	130	180
July 13	B-1	12.3	Thin	117	9.5	130	180
July 19	B-2	11.3	Thin	104	9.2	130	180
Oct. 17	C	11.6	Very thin	108	9.3	130	220
Oct. 4	D-1	11.9	Thin	118	10.0	134	180
Oct. 5	D-2	11.9	Thin	110	9.2	132	180
Nov. 16	D-3	12.3	Thin	124	10.0	112	154
Nov. 27	E	13.6	Thin	136	10.0	130	180
Nov. 8	F-1	13.2	Moderately fat	151	11.4	168	240
Nov. 9	F-2	13.2	Moderately fat	140	10.6	184	240
Oct. 29	G	13.8	Old and fat	193	14.0	174	240

* Since 30 ± 5 per cent of the nitrogen of the body was eliminated during the first 7 minutes of rinsing with oxygen, the values for total nitrogen and nitrogen per kilogram of body weight are only 70 ± 5 per cent of the total nitrogen present.

absorbed is proportional to the partial pressure of nitrogen in the lungs, then desaturation from 3 atmospheres of pressure should yield three times as much nitrogen as desaturation from 1 atmosphere, etc.; and if the rate of desaturation from 3 atmospheres of pressure is the same as the rate of desaturation from 1 atmosphere, then at all times the volume of nitrogen eliminated must be in the ratio of 3 to 1, etc.

Curve A, figure 2, represents the nitrogen desaturation from 1 atmosphere of pressure; curve B, desaturation from 3 atmospheres; and curve C, desaturation from 4 atmospheres. The curves are drawn through the experimental points which are indicated by 0, while the points indicated by x represent the nitrogen which would be eliminated if the volume

absorbed were exactly proportional to the increased pressure of the air breathed. Desaturation from 3 atmospheres of pressure yielded 334 cc. of nitrogen versus the theoretical value of 354 cc., giving a ratio of 2.83:1 instead of 3:1. Desaturation from 4 atmospheres yielded 500 cc. versus the theoretical value of 472, giving a ratio of 4.23:1 instead of 4:1. The discrepancies fall within the experimental error for this procedure. In another experiment of the same nature on dog C, it was found that desaturation from 4 atmospheres of pressure yielded 440 cc. of nitrogen and desaturation from 1 atmosphere of pressure yielded 108 cc. of nitrogen, the ratio being 4.14:1. These experiments indicate that the volume of

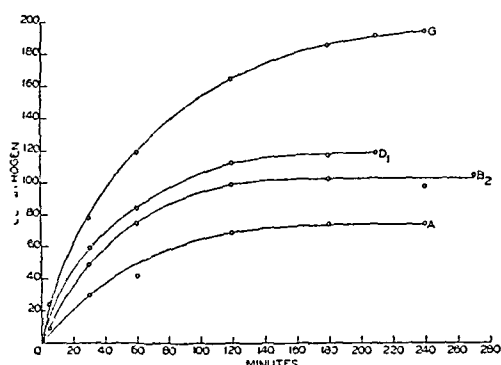


Fig. 1

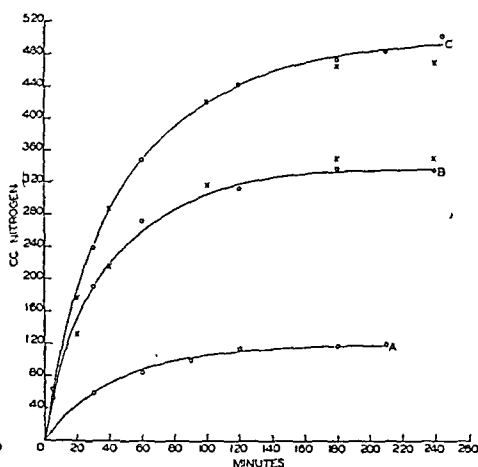


Fig. 2

Fig. 1. Desaturation from air at 1 atmosphere of pressure. The nitrogen eliminated during the first 7 minutes of oxygen inhalation was not collected.

Fig. 2. The nitrogen elimination of dog D saturated with air at 1, 3, and 4 atmospheres of pressure. The nitrogen eliminated during the first 8 minutes of oxygen inhalation was not collected. Curve A, desaturation from 1 atmosphere; curve B, desaturation from 3 atmospheres; curve C, desaturation from 4 atmospheres. \circ , experimental values; \times , theoretical values.

nitrogen absorbed is proportional to the partial pressure of the nitrogen in the lungs, and that the rate of desaturation from pressures of 1, 3 and 4 atmospheres is the same.

The nitrogen elimination curves following exposures to high pressures may throw some light upon the manner in which the excess nitrogen is held in the body. Is it held in physical solution or as minute bubbles? A gas which is held in physical solution in a fluid will tend to effervesce and form bubbles when the partial pressure of that gas exceeds the barometric pressure upon the fluid. At a pressure of 1 atmosphere, therefore, we should expect to find nitrogen leaving the blood as bubbles when its partial pressure exceeds 760 mm. When the bubble first forms the

partial pressure of the nitrogen in it, under normal atmospheric conditions, is 760 mm.; but as the gas in the bubble comes into equilibrium with the tissue gases—water, carbon dioxide and oxygen—the nitrogen becomes diluted until its partial pressure is approximately 627 mm. This pressure will remain constant so long as any excess nitrogen remains in the body. Under these conditions the nitrogen would pass from the tissues into the blood under a constant pressure head, and consequently at a constant rate, until the bubbles were completely absorbed. The resultant desaturation curve would tend to be a straight line whereas the true curve tends to flatten progressively, indicating a constantly diminishing yield of nitrogen per minute.

The curves presented, therefore, show that the body fluids are capable of carrying nitrogen in a state of supersaturation. Rapid decompression from 4 atmospheres of pressure results in a degree of nitrogen supersaturation of the tissues which may be measured by the difference between the initial nitrogen pressure of 2370 mm. existing in the body at the moment of decompression, and the barometric pressure of 760 mm. which immediately follows. The difference ($2370 - 760 = 1610$ mm.) is far too great to be explained by our present knowledge of the behavior of gases in supersaturation in vitro. We must, therefore, attribute this phenomenon to some characteristic of the body fluids which functions only in vivo.

To determine whether nitrogen passes into the tissues at the same rate that it is given up, when the pressure head is the same in both cases, the dog was completely desaturated and then exposed to air at different pressures for periods of time less than that required for complete saturation. If the total volume of nitrogen taken up during the saturation period is the same as that given off during the same period of time, then the saturation time equals the desaturation time. In figure 3, curve *A* represents the desaturation rate of dog *D* following a state of equilibrium with air at 1 atmosphere of pressure. The same dog, after complete desaturation, was then made to breathe air at 1 atmosphere for 67 minutes and again the nitrogen was collected by exposure to oxygen, as shown by curve *B*. If the unknown quantities of oxygen lost during the first 7 minutes in each experiment were approximately equal, so that the nitrogen collected in the succeeding 60 minutes was an indication of the relative rates of saturation and desaturation, then the ratio of saturation time to desaturation time is 92:86, or a difference in rate of only 7 per cent. This relationship is shown by the broken line. The same experiment was done under a pressure of 4 atmospheres, curve *C* representing the nitrogen collected after complete saturation and curve *D* the nitrogen collected after an exposure of 67 minutes. In this case the ratio is 348:368, or a difference in rate of about 6 per cent. This discrepancy is no greater than

we should expect to arise from the experimental errors, so that we may conclude that the saturation time and desaturation time are essentially equal.

Experiments were done to determine the desaturation time of the body following incomplete saturation with nitrogen. Dogs were first completely desaturated of the nitrogen in the body and then exposed to air for periods of time which were less than the known saturation time. The nitrogen from the partially saturated body was then collected by the procedure herein described and the desaturation curve constructed. One might

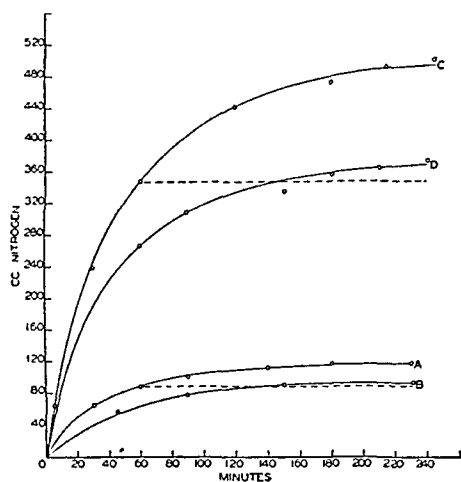


Fig. 3

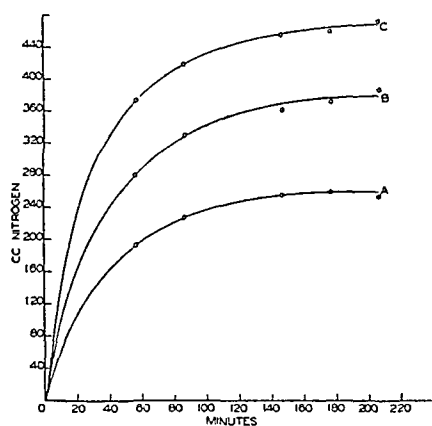


Fig. 4

Fig. 3. Saturation time compared with desaturation time. Dog D. The nitrogen eliminated during the first 7 minutes of oxygen inhalation was not collected. Curve A follows complete saturation at 1 atmosphere; curve B follows 67 minutes' saturation at 1 atmosphere; curve C follows complete saturation at 4 atmospheres; curve D follows 67 minutes' saturation at 4 atmospheres.

Fig. 4. The nitrogen elimination after incomplete saturation with air at 3, 4, and 5 atmospheres. Dog E. The nitrogen eliminated during the first 8 minutes of oxygen inhalation was not collected. Curve A follows 65 minutes' saturation at 3 atmospheres; curve B follows 65 minutes' saturation at 4 atmospheres; curve C follows 65 minutes' saturation at 5 atmospheres.

expect under these conditions that the nitrogen from the rapidly saturating tissues would be released very rapidly, and that the curve would then flatten out with extreme abruptness due to the fact that the slowly saturating tissues have been only partially saturated. According to this hypothesis, the shorter the period of saturation, the greater should be the departure from the normal desaturation curve as determined under equilibrium conditions. Our experiments show, however, that the desaturation time is constant for all degrees of previous saturation. Inspection of figure 4 will make this apparent. Curves A, B and C represent the nitrogen

desaturation time of dog E after exposure to 3, 4 and 5 atmospheres of pressure for 65 minutes. The time of desaturation was not 65 minutes but 200 minutes, the latter being the time which was required for this particular dog to give up its nitrogen after complete saturation. It is interesting to note that the shape of these curves resembles, in all respects, the desaturation curves of dogs which have been completely saturated.

That the desaturation time is constant, irrespective of the degree of saturation, may be more accurately demonstrated by curves which correlate per cent desaturation with time. The curve in figure 5 shows the rate at which the nitrogen is eliminated from dog D, when desaturated from a state of equilibrium with air at 1 atmosphere of pressure, in terms of percentage desaturation against time. When the same dog was exposed to

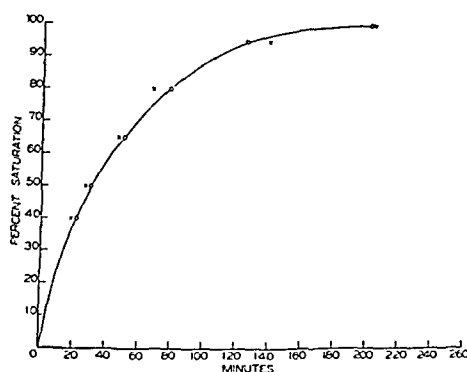


Fig. 5. The nitrogen elimination after incomplete saturation with air at 4 atmospheres. Dog D. The nitrogen elimination during the first 8 minutes of oxygen inhalation was not collected. O, desaturation rate following complete saturation. X, desaturation rate following partial saturation.

4 atmospheres of pressure for 37 minutes and the desaturation rate then reduced to the same terms, it is found that the points (x) for this curve coincide with the curve for complete saturation.

The fact that the rate of nitrogen elimination from the partially saturated body coincides with that of the completely saturated body indicates that the nitrogen in the quickly saturating tissues is constantly moving into the more slowly saturating tissues, thus tending to equalize the nitrogen tension throughout the entire body at all times. For example, at normal atmospheric pressure, about 120 cc. of nitrogen were given off by dog D when completely desaturated under the conditions of our experiments. When exposed to 4 atmospheres of pressure for 37 minutes the same dog under the same experimental conditions gave off 266 cc. of nitrogen. This curve is exactly similar to that which we should expect to find if the dog had been completely saturated at an atmospheric pressure which was proportional to the nitrogen eliminated, or 2.22 atmospheres

(266/120 = 2.22). Had there been a tendency for the nitrogen tension in the rapidly saturating tissues to remain in equilibrium with the tension of nitrogen inspired, i.e., air at 4 atmospheres, while the more slowly saturating tissues were still unsaturated, then the first part of the curve should have been disproportionately steep. These findings lead to the conclusion that complete desaturation from a state of partial saturation follows the same curve, and consequently requires the same length of time as desaturation from a state of complete saturation.

SUMMARY

1. A method for measuring the nitrogen content of the body and its rate of elimination is described. The experiments were done upon dogs.

2. Under conditions of equilibrium at pressures up to 4 atmospheres absolute, the nitrogen content of the body is proportional to the partial pressure of nitrogen in the lungs, and the rate of nitrogen elimination is the same at all pressures.

3. The rate of nitrogen elimination from the completely saturated body, i.e., under conditions of equilibrium, is the same as the rate of elimination from the partially saturated body.

4. The saturation time is the same as the desaturation time.

5. Evidence is given showing that nitrogen is held by the blood and tissue fluids in a state of supersaturation to a high degree.

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THE PSYCHOLOGIC EFFECTS FROM BREATHING AIR AT 4 ATMOSPHERES PRESSURE¹

ALBERT R. BEHNKE,² ROBERT M. THOMSON AND E. PREBLE MOTLEY
From the Department of Physiology, Harvard School of Public Health, Boston, Mass.

Received for publication March 13, 1935

Air at high barometric pressures produces a narcotic effect on man. The changes which first appear at 3 atmospheres pressure are expressed in alterations of behavior, slowed mental activity, and impaired neuromuscular coördination. These effects, although previously observed, have not been attributed to the direct action of air or of the component gases, oxygen, nitrogen³ and carbon dioxide.

Hill and Phillips (1932) reported that some of the deep sea divers (at depths of 270 to 300 feet, or 9 to 10 atmospheres absolute) had difficulty in assimilating facts and in making quick decisions, summarized as "a slowing of the process of cerebation." Severe emotional disturbances were sometimes observed and loss of consciousness reported. The men affected in this manner were regarded as psychologically unstable. Damant (1930) with reference to the paper of Hill and Phillips mentioned the "subtle change in character and behavior which comes over some men at less high air pressures," and considered that either the increased oxygen tension or impurities in the air might be responsible. Hill, according to Damant, excluded oxygen and carbon dioxide as etiologic factors.

Because of the many abnormal factors in the environment of the deep sea diver, the conclusion could not be drawn that changes in behavior and mental activity were caused by increased air pressure alone. It was of interest, therefore, to make observations on persons who were subjected to high air pressures in surroundings that were otherwise normal.

This paper presents a description of the psychic changes in air at 4 atmospheres, and the inference is drawn that the increased partial pressure of nitrogen is mainly responsible for the abnormal responses.

The observations were made in a large, well lighted pressure chamber described by Thomson, Yaglou and Van Woert (1932), on nine persons engaged in physiologic research under ordinary laboratory conditions except that the barometric pressure was raised from 1 to 4 atmospheres.

¹ This research was aided by the Miriam Smith Rand Fund.

² Member of the United States Naval Medical Corps.

³ Includes argon and the rare gases.

The time of exposure to the increased pressure varied from 1.5 to 5 hours including decompression time. The temperature of the air was 25°C. and the relative humidity 50 per cent. Variations in temperature and humidity did not occur except for a period of 10 minutes during compression from 1 to 4 atmospheres and for a period of 2 minutes during decompression from 4 to 2.3 atmospheres.

The evaluation of the psychic changes was made on the basis of subjective and objective reactions of trained men engaged in animal experimentation. The usual procedures followed were cannulation of blood vessels, withdrawal of blood and cisternal fluid, collection of alveolar gas samples, and recording the blood pressure and oxygen consumption. Quantitative values have not been assigned to the changes, and their validity rests on the basis of a constant altered response of trained men when the air pressure was raised from 1 to 4 atmospheres.

OBSERVATIONS. The abnormal reactions were first felt at a pressure of 3 atmospheres. At 4 atmospheres the members of the entire group were affected in a similar manner with differences only in the degree of intensity of the responses.

Emotional reactions. As a pressure of 4 atmospheres was approached the individual was definitely aware of a feeling of stimulation, alertness and well-being—a definite euphoria. The mood was usually well controlled but occasionally expressed itself in laughter and loquacity. With a greater effort of self-control, normal conduct could be maintained.

Impairment of the higher mental processes. A slowing up of mental activity was a characteristic response. Visual, auditory, olfactory and tactile reception were not affected, but the response to these stimuli was delayed. A limitation of the power of association and a tendency toward fixation of ideas had to be counteracted. Recollection, consequently, required greater effort, and concentration was comparatively difficult. Frequent errors were made in arithmetical calculations and in the recording of data. One person had difficulty in telling time, confusing 43 minutes, for example, with 48 minutes. In the recording of time a reading of 12:15 might be written as 15:15. The responses were those of mild stupor in which greater effort and more time were necessary for accuracy.

Impairment in neuromuscular control. Increased difficulty attended the measurement of liquids and the manipulation of burettes and pipettes. The turning of stopcocks in the wrong direction was frequent. The impaired control of finer movements increased the breakage of glassware. The defect in coördination was essentially a past pointing or exaggeration of movement. The impairment, however, became negligible if slower movements were made.

In a single test at a pressure of 10 atmospheres (in another chamber) one member of the group felt a greatly increased numbness which amounted

to partial stupefaction. A simple task, the palpation of the pulse of another worker, was accomplished only with extreme difficulty. Efficient neuromuscular response was abolished.

That the changes increase in severity as the pressure is increased is shown by the responses at 3, 4, and 10 atmospheres. The retardment of mental activity is first felt at 3 atmospheres, becomes a slight handicap at 4 atmospheres, and at 10 atmospheres may render an individual helpless.

The altered responses occurred at the beginning of an exposure to increased pressure, and they did not change in intensity over periods of 3 hours. Irritability, fatigue and drowsiness were not present, but following decompression fatigue with a tendency to sleep was the usual reaction. Since this fatigue can be abolished by breathing oxygen during decompression, it is probably related to the presence of excess nitrogen possibly in the form of small bubbles.

DISCUSSION. Nature of the changes. The altered responses in air at high barometric pressures can be produced also by a variety of agents which depress the higher centers. Among these are alcohol, the inhalation anesthetics, i.e., ether, nitrous oxide, narcotic drugs, and conditions which lead to anoxemia. Thus the mental responses from decreased oxygen tension at normal barometric pressure (McFarland, 1932), and at moderately high altitude (Barcroft, 1925) are similar to the responses at increased air pressures.

Since the severity of the reactions is related to the degree of pressure and ranges from slight impairment at 4 atmospheres to partial stupefaction at 10 atmospheres, it is inferred that a limiting pressure incompatible with human activity would be reached between 10 and 15 atmospheres pressure.

The consideration of the etiology of the responses associated with high air pressures brings into the discussion the effects of the increased tensions of oxygen and nitrogen.

The action of the increased partial pressure of oxygen. At 4 atmospheres the partial pressure of oxygen in the air (4×21 per cent) is 84 per cent of 1 atmosphere, and at 10 atmospheres of air is equivalent to 2.1 atmospheres of pure oxygen. The breathing of pure oxygen, however, at pressures of 1 to 4 atmospheres does not induce the psychic responses of air with corresponding or considerably lower oxygen tensions (Behnke, Johnson, Poppen and Motley, 1935). Oxygen breathed for a period of several hours may disturb the coördination of finer movements, but euphoria is not present. In contrast with the effect of oxygen, the symptoms at high air pressures are immediate in their onset. The increased partial pressure of oxygen, therefore, cannot be a significant factor in the etiology of the changes.

*The action of the increased partial pressure of nitrogen.*⁴ If oxygen is

⁴ Includes argon and the rare gases.

excluded, then the atmospheric nitrogen can be considered as mainly responsible for the narcotic action of air at high pressures. Although nitrogen is chemically inert, the physical property which renders this gas analogous to narcotic substances is its high coefficient of solubility in lipid matter. Ether and nitrous oxide which induce narcosis are also chemically inert substances. The Meyer-Overton law indicates that a definite relationship exists between the power of a narcotic (derivatives of the hydrocarbon series) and its "partition coefficient," or the ratio of the solubility in fat to the solubility in water. Although the Meyer-Overton law was formulated with regard to the aliphatic narcotics, it is not unreasonable to assume that the high concentration of molecular nitrogen in the lipoids of the central nervous system decreases cell membrane permeability to produce the changes characteristic of air at high pressures.

The "partition coefficient" of ether is 4.3, that of nitrous oxide 1.89, and that of nitrogen 5.0. Nitrous oxide induces anesthesia when the concentration in the blood is 0.017 mol per liter, and ether acts in the same manner when its concentration is 0.003 mol per liter. At an air pressure of 10 atmospheres the molal concentration of nitrogen in the blood is 0.0043, and at a pressure of 40 atmospheres 0.017 mol or the equivalent of the anesthetic concentration of nitrous oxide. While the limiting pressure at which air is respirable has not been determined, it is believed that loss of consciousness might occur between 10 and 15 atmospheres pressure.

PRACTICAL CONSIDERATIONS. An artificial gas mixture for divers is essential if operations at great depths (above 300 feet) are carried out, and for caisson workers if the exposure at and higher than 4 atmospheres be continued over long periods of time. Such a mixture, of course, should limit the oxygen concentration to that in the air at sea level, and in addition should provide a rapidly diffusible, sparingly soluble gas with a low "partition coefficient." The last qualification is of the greatest importance since large quantities of gas dissolved in fat relative to the amount dissolved in water increase the danger of bubble formation on decompression. Furthermore, if our hypothesis be correct, the psychic effects produced by a gas like nitrogen with its high solubility coefficient in fat, will be minimized.

SUMMARY AND CONCLUSIONS

Air at and higher than 3 atmospheres pressure exerts a narcotic effect on man with the characteristics of euphoria, retardment of the higher mental processes, and impaired neuromuscular coördination.

At 4 atmospheres these changes can be counteracted by increased effort, but at 10 atmospheres they amount to stupefaction with greatly impaired muscular activity.

The increased partial pressure of oxygen does not account for these changes. It is inferred that the atmospheric nitrogen is the etiologic

factor, and that it acts on the nervous system because of the high solubility coefficient of this gas in lipoid substances compared with that in water.

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OBSERVATIONS ON THE VARIATION IN WATER CONTENT OF THE FECAL MATERIAL ALONG THE COLON

F. R. STEGGERDA

From the Department of Physiology, College of Medicine, University of Tennessee, Memphis, Tennessee

Received for publication April 10, 1935

It is generally accepted that the concentration of fecal material due to absorption of water takes place in the cecum and proximal colon. Roith (1) states that "The contents of the transverse colon in man are generally as firm as those in the rectum." Larson (2) in his review of the physiology of the colon, points out that "if fluids went beyond the hepatic flexure, they would interfere with the storage function of the colon." Hurst (3) asserts that while most of the water absorption takes place in the cecum and ascending colon, and very little in the transverse and descending colon, there is further absorption in the pelvic colon, which is responsible for the normal consistency of the feces. Considerable has also been written concerning the nature of the absorption and excretion of various substances by the colon, especially with reference to different concentrations of salts, sugar, heavy metals and drugs (4, 5, 6, 7, 8). Most of these investigations were made by means of isolated colon pouches, or previously purged colons, and sometimes with the animal under some type of anesthetic.

To our knowledge, little or no work has been done concerning the actual percentage differences in the water content of fecal material in the cecum, colon and rectum in apparently normal animals. Therefore, we undertook the investigation of this problem by determining the water content of the feces of the dog at different parts of the large intestine—namely, the cecum, proximal and distal colon, and rectum. Data will also be presented concerning the water content of the feces lodged in the distal colon and rectum when the contents of the small intestine are shunted directly into the distal colon. Finally we shall present a table showing how the water content of different parts of the human stool varies similarly to that of the dog.

Under anesthesia and by aseptic technique two fistulae were made in the colon of dogs, one in the cecum and another in the proximal colon, about 10 cm. distal to the ileo-cecal valve. The patency of the fistulae was maintained by a cannula made of a light weight hard rubber fiber about 4 cm. long, with an inside bore of 1.5 cm. The end inserted into the lumen of the colon had a flange of 1 cm. in width to insure retention of the cannula.

The muscular wall of the colon was ligated around the neck just above the flange. The other end of the cannula was brought to the outside through a stab wound in the abdominal wall, and was held in place by a rubber washer threaded on the cannula. The fecal material was prevented from entering the lumen of the cannula by a fiber plug of the same length and diameter as the inside bore of the cannula. This plug was held in place by a rubber screw cap. By means of these short cannulae we were able to take 2 or 3 gram samples of fecal material directly from the lumen of the cecum and colon by using a special curette. In later experiments we substituted for the hard rubber cannula a large pezzet catheter made of softer rubber, from which we cut the lower half of the bulb, the remaining half serving as a flange. This type of tube proved much more satisfactory. The permanent fistula devised by Mann and Bollman (9) in which a piece of reversed small intestine about 8 inches long is connected between the colon and abdominal wall, was also tried; this we found unsatisfactory, because a true sample of concentrated fecal material could not be obtained.

Each animal was given careful post-operative care, and after a week or ten days the colon had apparently regained its normal tone and function. The dogs were healthy and active, and on a standard diet of meat, bread and milk, passed stools of normal consistency. After the dogs had reached this point, we collected samples almost every morning from the cecum and proximal colon through the two fistulae, directly into previously weighed weighing bottles. Shortly after the samples had been collected, the dogs were allowed to exercise, and with surprising regularity they passed stools from 6 to 8 inches long within 15 minutes. We then collected 5 to 10 gram samples from the two ends and middle part of this stool, assuming that this material had just previously been in contact with the mucosa of the rectum, pelvic colon, and possibly the descending colon (Hurst (3) reports that upon defecation the colon may at times be emptied as high as the splenic flexure). All these samples were dried to a constant weight of 100°C. and the percentage water content calculated.

Hurst (3) and other early investigators believed, and physiology textbooks assert, that feces do not accumulate in the rectum until shortly before the desire to defecate. The opposite view is upheld by Alvarez (10) and other clinicians who find that the rectum is full of feces long before there is a desire to defecate. This fact was confirmed on different occasions by roentgenologic studies when after a barium meal the rectum of the dog, as well as that of man, was found to be full several hours before defecation took place. We also found that the first 2 inches of the stool were far more concentrated than the rest of the stool—this concentrated portion corresponds to the approximate length of the dog's rectum.

The average percentage water content of the samples from the different parts of the large intestine in one particular dog is presented in figure 1.

It was found that the average water content of the cecal samples was 87 per cent, while that of the proximal colon samples, 10 cm. distal to the ileocecal valve, was 10 per cent less. This difference clearly indicates that a considerable concentration of fecal material takes place in the cecum. The samples taken from the end and middle sections of the stool, those in contact with the descending and pelvic colon respectively, show a difference in concentration of only 4 per cent from the proximal colon sample—a very

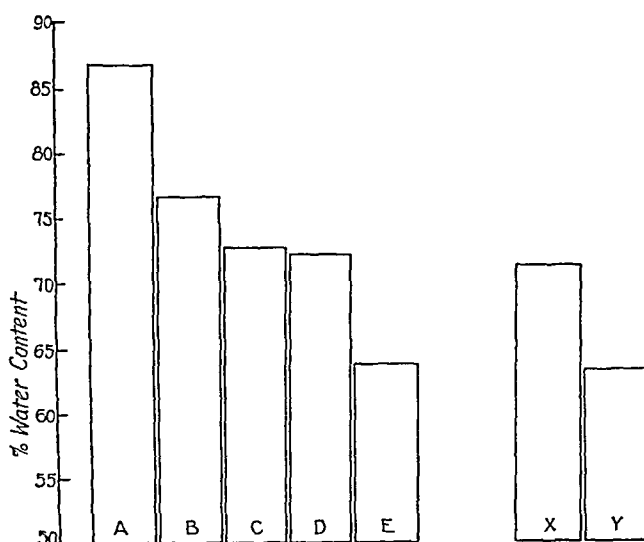


Fig. 1. Percentage water content of the fecal material in the colon of the dog. A. Average percentage water content of 10 samples of fecal material collected from the cecal fistulae.

B. Average percentage water content of 9 samples of fecal material collected from the fistulae in the proximal colon.

C. Average percentage water content of 13 samples taken from the end portion of the stool.

D. Average percentage water content of 10 samples taken from the middle portion of the stool.

E. Average percentage water content of 13 samples taken from the rectal portion of the stool.

X.Y. Average percentage water content of 26 samples taken from the last and rectal ends of the stool, after the small intestine was anastomosed to the lower colon.

The percentage water content of all the samples in this curve was collected from the same animal.

slight one considering the length of the colon traversed. The sample taken from the first section of the stool, however, that in contact with the rectal mucosa, shows another marked water loss of nearly 10 per cent.

These results indicate that the rectum and cecum play a major part in the concentration of fecal material. This may be explained by the fact that the cecal and rectal portion of the large intestine have a much richer blood supply than the remaining part of the colon—a fact which we con-

firmed by actually outlining the vascular supply to the large intestine with injection material immediately after death from hemorrhage.

Considering the nature of these results, we were interested to learn the effects of shunting the contents of the small intestine directly into the descending colon, and thereby preventing the cecum from performing its normal part in the concentration of the feces. A second operation was therefore performed on the same animal, in which the ileum was sectioned near the ileocecal valve and closed. The ileum was then anastomosed laterally to the descending colon about 8 cm. below the splenic flexure. After the recovery of the dog, using the same diet as before, we again collected and dried samples of the stools, from the rectal and colon ends only, since the middle was so nearly like the colon end. We found that the dif-

TABLE 1
Water content of samples of human feces

NUMBER	NAME	DATE	RECTAL	MIDDLE	END
1	F. R. S.	Oct. 31	69.77	81.34	82.00
2	F. R. S.	Nov. 1	65.33	78.08	78.24
3	F. R. S.	Nov. 4	71.12	82.79	81.13
4	F. R. S.	Nov. 5	72.55	85.31	85.13
5	F. R. S.	Nov. 7	72.25	79.96	82.03
6	F. R. S.	Nov. 8	71.50	82.04	84.17
7	F. R. S.	Nov. 10	74.81	82.34	85.58
Average.....			71.05	81.69	82.61
H. H. M.	Average (5 stools)		62.60	73.18	72.24
C. D.	Average (6 stools)		72.56	80.03	82.24
D. P.	Average (6 stools)		64.81	71.63	72.40

ference in water content in the two ends of these stools was practically identical with the percentage difference in those collected from the two ends before the operation (see figure 1). At first we thought the rectum must be responsible for the main part of this concentration. However, it happened in the course of our experiments that we had one dog in which the cecal cannula was retained for four weeks after the ileum was anastomosed to the descending colon, so that in this dog we were able to continue the collection of cecal samples; and we found that the fecal material had in some manner been pushed back through the proximal colon into the cecum, where normal absorption could take place. We confirmed this on several occasions by adding carmine dye to the afternoon feeding of the dog. The next morning the dye showed up very distinctly in the cecal samples collected. Two days later no trace of carmine dye could be found

in the samples. This might indicate that some anti-peristaltic force tends to push fecal material back into the cecal region for concentration (11, 12, 13). On the other hand, the forward movement of the small intestine, combined with the pressure of the fecal material, might cause such an accumulation without the aid of anti-peristalsis.

Although it is common knowledge to anyone familiar with analyzing the human stool that the portion considered to have been in contact with the rectum is more solid than the rest of the stool, it was of interest to find the percentage difference in water content of the human stool, and whether the rectum has the power of concentrating the feces as it has in the dog. Five to seven stools were collected from four different persons, and samples were taken from the first, middle, and last portion of each stool and dried to constant weight in previously weighed weighing bottles (table 1).

The results proved to be very similar to those found in the dog; the first portion, which we assumed on the basis of previous roentgenologic studies to be in contact with the walls of the rectum was about three inches long (which is the approximate length of the rectum) and was about 7 to 12 per cent more concentrated than the portion just proximal to it. The difference between the middle and proximal portions was very slight—about 1 per cent.

It should be pointed out that all this work on both human and dog feces was carried out when no sign of constipation or diarrhea was evident.

CONCLUSIONS

1. The percentage water content of fecal material at points along the entire colon was determined. It was found that the feces in the proximal colon are 10 per cent more concentrated than that in the cecum, and that the rectal end of the stool is 10 per cent more concentrated than the part lodged in the lower colon.

2. There is a direct relation between the concentration of feces in the cecum and rectum and the blood supply to these parts.

3. When the contents of the small intestine are emptied directly into the descending colon by means of ileo-colostomy, there is evidence of some force which pushes the fecal material back into the cecum where it is concentrated as it was before the operation.

4. The percentage difference in the water content of the various parts of the human stool is similar to the percentage difference in that of the dog.

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CARBOHYDRATE CHANGES DURING RECOVERY FROM MUSCULAR CONTRACTION

JACOB SACKS AND WILMA C. SACKS

*From the Laboratory of Pharmacology, University of Michigan Medical School,
Ann Arbor, Michigan*

Received for publication April 12, 1935

Skeletal muscle has two mechanisms available for disposing of the lactic acid that accumulates during contraction. One is the well known reaction described by Meyerhof (11), by which the major part of the lactic acid is converted to glycogen within the muscle at the expense of the oxidation of the smaller part to carbon dioxide and water. This reaction has been demonstrated by Meyerhof in isolated frog muscle, and has been shown to take place in isolated dog muscle by Shorr, Loebel and Richardson (16), and in isolated rabbit and beef muscle by Boyland (1).

The other mechanism involves the muscle-liver cycle of lactic acid and glucose first proposed by Himwich, Koskoff and Nahum (8). According to this, the lactic acid of the muscle diffuses into the blood stream and is removed from the blood by the liver, where it is converted to glucose which is in turn carried to the muscles and there converted to glycogen.

It becomes of considerable importance, in connection with theories of the source of energy for contraction, to ascertain the relative quantitative importance of these two mechanisms. If the "Meyerhof cycle" is shown to be the predominant one, then the findings of Owles (13), and of Margaria, Edwards and Dill (10), among others, that in light or moderate exercise little or no lactic acid appears in the blood, is easily explained on the basis of either the original Hill-Meyerhof theory or that of Lundsgaard. However, if it can be shown that the muscle-liver cycle predominates, then the findings can be explained only on the basis that the energy-yielding reactions are primarily oxidative, that the appearance of lactic acid in the blood during exercise is evidence of inadequate oxygen supply, and that the anaerobic mechanisms are but stop-gaps during the period of circulatory adjustment.

Eggleton and Evans (5) have shown that large amounts of lactic acid do enter the blood stream within a very short time after periods of violent muscular contraction, that the removal of this lactic acid is a slow process, and that the liver is responsible for the removal of the major part of the lactic acid. They state that lactic acid enters the blood stream from the

stimulated muscles throughout the entire recovery period. Although this work tends to show that the cycle involving the liver is the more important one, the nature of their experimental procedures was such that it was impossible to evaluate the quantitative importance of this mechanism relative to the "Meyerhof cycle" within the muscle.

The present work was undertaken to determine the relative importance of the two mechanisms. The procedure used is based on the following propositions: if, in a group of animals of one species which are of uniform age and weight, a certain muscle is stimulated isometrically for a definite, constant time, the amounts of lactic acid and of hexosephosphate formed, and of glycogen lost, should lie within a relatively narrow range. Now, if in another group of animals, similar to the first, the same muscle is stimulated under the same conditions, it would be expected that the same quantities of lactic acid and hexosephosphate would be formed, and glycogen lost, as in the first series. If the muscles in the second group are not analyzed at the end of the stimulation period, but are allowed to recover for a definite time and then submitted to analysis, the differences in the average values for the two groups can be held to give a reasonably accurate picture of the changes which have taken place during the recovery period. If it is found that the lactic acid loss from the muscle during the recovery period is greater than the net loss of total carbohydrate, this would show that a large part of the lactic acid had been converted to glycogen within the muscle. On the other hand, if the net loss of glycogen in the recovery period approaches the loss of lactic acid, it would show that diffusion into the blood stream accounted for the removal of the major part of the lactic acid. Of course, this does not permit any differentiation to be made between the lactic acid lost by oxidation and that lost by diffusion. However, it will be seen from the data that the changes are of such magnitude that except toward the end of the recovery period, there can be no doubt that oxidation could be of only minor quantitative importance in the removal of lactic acid.

The experiments were performed on rabbits which were about 3 months old and weighed from 1250 to 1500 grams. Under amytal-ether anesthesia, one gastrocnemius muscle was tetanized isometrically for 30 seconds, care being taken to leave the blood and nerve supply intact. The recovery periods used were 5, 10, 20 and 30 minutes. The muscles which were not allowed to recover were frozen within a few seconds after the end of the stimulation period. The resting muscle was frozen as soon as possible after the one which had been stimulated, generally within one minute. Determinations of lactic acid content, hexosephosphate formation and phosphocreatine hydrolysis were made by the methods used previously (14). Glycogen was determined by digestion with 30 per cent KOH, precipitation according to the method of Good, Kramer and Somogyi (7),

TABLE 1—*Concluded*

RESTING MUSCLE				STIMULATED MUSCLE				DIFFERENCE			CARBOHY- DRATE BALANCE
Inor- ganic P	Inor- ganic plus phos- pho- creatine P	Lactic acid	Gly- cogen	Inor- ganic P	Inor- ganic plus phos- pho- creatine P	Lactic acid	Gly- cogen	Phos- pho- creatine hydroly- sis	Hexose- phos- phate forma- tion	Lactic acid	
20 minutes' recovery											
17	96	25	645	18	95	33	370	1	1	8	-261
20	97	18	980	17	90	38	660	-3	7	20	-260
20	100	19	1270	16	88	50	1025	-4	12	31	-144
14	85	37	915	14	79	58	610	0	6	19	-249
15	104	25	620	13	88	53	265	-2	16	28	-233
Average.....								-2	8	21	-229
Carbohydrate loss.....229											
Lactic acid loss.....138											
30 minutes' recovery											
17	92	22	520	17	87	26	265	0	5	4	-212
18	106	23	920	17	106	33	605	-1	0	10	-305
20	95	19	745	20	95	27	480	0	0	8	-257
19	90	13	655	17	79	20	545	-2	11	7	-39
Average.....								-1	4	7	-203
Carbohydrate loss.....203											
Lactic acid loss.....152											

hydrolysis of the precipitated glycogen with N/2 HCl, and determination of the reducing value of the neutralized hydrolysate with the Shaffer-Somogyi reagent 50 (15). Glycogen values in the tables are in terms of glucose equivalent.

There is a distinct advantage in stimulating a single muscle rather than the entire musculature of both hind legs, as was done by Eggleton and Evans. As the total quantity of lactic acid formed under the present conditions is small, it can be considered to be diffusing into blood which has a constant lactic acid content throughout the entire recovery period, for the load placed on the liver in removing this small amount of lactic acid is a light one.

Carbohydrate balances were drawn up in this way: For the resting muscle the sum of the lactic acid and glycogen contents was taken; for the stimulated muscle there was added to the sum of these values the glucose equivalent of the hexosephosphate difference between the two muscles. This glucose equivalent is obtained by multiplying the difference

between the sums of inorganic- and phosphocreatine-P of the resting and stimulated muscles by 5.8, the ratio between the equivalent weights of glucose and P.

Examination of the data shows that, while variations in the total carbohydrate content do appear between the two corresponding muscles of a single animal, the differences tend to disappear if several animals are considered together. In the group with no recovery the average net loss of carbohydrate is only 24 mgm. per cent. Although this small difference may be within the limits of experimental error, there are good reasons for regarding it as significant. For one thing, Cori, Closs and Cori (2) have shown that a certain amount of glucose is formed during a tetanus, the probable source of which is glycogen. Also, it is not unlikely that a small amount of lactic acid does diffuse into the blood stream during the tetanus. Both of these processes would result in an apparent net loss of carbohydrate in the present experiments, since muscle sugar determinations were not made.

During the first five minutes of recovery it is seen that a very considerable part of the lactic acid is lost from the muscle. On the basis of the "Meyerhof cycle" some part of this lactic acid should have been converted to glycogen within the muscle and the net loss of carbohydrate should be less than the loss of lactic acid. However, there is an additional net loss of carbohydrate over and above the decrease in lactic acid content. Throughout the entire recovery period, until the lactic acid content returns practically to the resting level, this condition persists; the loss in total carbohydrate is always greater than the loss of lactic acid.

It thus becomes evident that there is no detectable conversion of lactic acid to glycogen within the muscle recovering from contraction. It is thus seen that diffusion into the blood stream is the mechanism utilized by the muscle for disposing of lactic acid not oxidized. The "Meyerhof cycle" cannot be considered to play any appreciable rôle in the recovery process in mammalian muscle. Insofar as lactic acid removal is concerned, the liver must be regarded as the organ which accomplishes recovery from anaerobic work.

It is necessary to account for the extra loss of carbohydrate which becomes evident early in the recovery period. The phenomenon of "delayed lactic acid formation" can account, doubtless, for a considerable part of this extra carbohydrate loss. The lactic acid formed in this way also diffuses into the blood and the actual quantity so lost is greater than the observed difference in lactic acid content between the stimulated and recovering muscles. In addition, muscle is known to have an increased oxygen consumption above the resting rate during the period of recovery after contraction. The oxidation of carbohydrate by this extra oxygen probably accounts for the remaining portion of the loss.

There has been evidence available for some years that mammalian muscles do not convert injected lactic acid to glycogen. Elias and Schubert (6) found as early as 1918 that the intra-arterial injection of lactic acid did not result in glycogen deposition in the muscles of dogs. Janssen and Jost (9) were unable to find any evidence of glycogen deposition in the muscles following intravenous injection of lactic acid. It is only in isolated muscles that the "Meyerhof cycle" has been found to take place. In this connection it may be pointed out that Eggleton and Evans were unable to confirm the findings of Meyerhof, Lohmann and Meier (12), that perfusion of frog muscles with solutions containing lactate leads to deposition of glycogen.

The resynthesis of glycogen from hexosephosphate seems to proceed at a rather constant rate. Cori and Cori (3) found in experiments on rats that about 1 mgm. per cent of hexosephosphate-P per minute was converted to glycogen during the recovery period. This is approximately the rate found in the present experiments during the early part of the recovery period. The rate does apparently fall off later on. This falling off may be considered as mass action effect. As the concentration of hexosephosphate present decreases, the rate at which it is converted to glycogen could be expected to decrease. With regard to the path by which this substance is converted to glycogen, the suggestion is made by Cori and Cori that the immediate fate is conversion to lactic acid which then undergoes the Meyerhof reaction. They have shown that under anaerobic conditions hexosephosphate is converted to lactic acid, but it is evident from what has been said above that this reaction does not take place in the presence of oxygen, for any lactic acid so formed would escape into the blood and be lost to the muscles. Hence the conclusion is drawn that hexosephosphate is converted directly to glycogen by the reversal of the reactions leading to the formation thereof.

Examination of the values for inorganic phosphate in the muscles which recovered for 5 or 10 minutes shows that they are definitely lower than those for the corresponding resting muscles. This finding bears out the interpretation previously placed upon the function of the hydrolytic reaction of phosphocreatine (14). During the first 10 minutes of recovery the loss of lactic acid by the muscle is greater than the loss of base resulting from resynthesis of the phosphocreatine which had been hydrolyzed or converted to hexosephosphate during contraction. Hence the pH of the muscle fiber would be shifted to the alkaline side, were not some mechanism employed to remove additional base. The formation of phosphocreatine from some of the P which is normally present as inorganic phosphate would result in such a removal of base. The creatine necessary would be furnished by that liberated in the formation of hexosephosphate during contraction. Then, toward the end of the recovery period, as base is removed

by the conversion of hexosephosphate to phosphocreatine in greater measure than lactic acid is removed, this extra phosphocreatine is hydrolyzed. It is inadvisable to attempt any quantitative treatment of these data, but the shifts between inorganic phosphate and phosphocreatine that take place during contraction and recovery are invariably in the direction indicated by the requirement for maintaining a constant pH within the muscle fiber.

The question may properly be raised whether amytal or ether interferes with the resynthesis of muscle glycogen. There is no very great probability that they do. Eggleton and Evans found no difference between amytalization and decerebration with regard to the rate at which the muscles accumulate glycogen during the recovery period, and Debois (4) obtained resynthesis at approximately the same rate under ether that Eggleton and Evans did under amytal and in decerebrate animals. Since neither drug by itself interferes with this process when it is the sole anesthetic, there is no reason to assume that the two together will have such an effect.

The data presented here offer a satisfactory explanation of the findings of Owles and of Margaria, Edwards and Dill that in light or moderate exercise no lactic acid appears in the blood. Under their conditions the oxygen transporting mechanism was adequate to meet the demands placed upon it; the work performed was done by means of oxidative reactions and no appreciable amount of lactic acid was formed in the muscles. If the rate of work is such that the circulatory adjustment can be accomplished rapidly and completely, the small amount of lactic acid formed and not oxidized would be so rapidly disposed of by the liver that it would not be detected; only if the circulatory adjustment lags will there be any accumulation of lactic acid in the blood.

SUMMARY AND CONCLUSIONS

1. The entire amount of lactic acid formed during anaerobic activity in muscle diffuses into the blood stream during recovery.
2. Glycogen is not formed in the muscle during recovery from any of the lactic acid produced in anaerobic activity.
3. The "Meyerhof cycle" is shown to be of no significance in recovering mammalian muscle.
4. Hexosephosphate which is formed during contraction is resynthesized directly to glycogen during recovery, without intermediate breakdown to lactic acid.
5. The rate at which hexosephosphate is resynthesized to glycogen is practically constant throughout the recovery period.
6. Additional evidence is presented that the sole function of phosphocreatine hydrolysis in muscle is the maintenance of constant pH within the fiber.

7. The data confirm the theory that the reactions which yield energy for muscular activity are primarily oxidative.

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THE AMERICAN JOURNAL OF PHYSIOLOGY

VOL. 112

AUGUST 1, 1935

No. 4

THE VISCERO-PANNICULAR REFLEX

D. M. ASHKENAZ AND E. A. SPIEGEL

From the Department of Experimental Neurology, D. J. McCarthy Foundation, Temple University School of Medicine, Philadelphia, Pa.

Received for publication April 13, 1935

In the course of experiments on visceral pain, we observed a reaction which seems quite valuable for the study of the conduction of centripetal impulses from the viscera. This reaction can be very easily produced and observed. It is very constant and it is present even when other reactions accompanying visceral stimulation can no longer be elicited.

The first observation was made in a decerebrate cat in which a balloon was tied into the gall bladder. This balloon was connected by a T-tube to a manometer and a rubber bulb for producing pressure. On dilatation of the gall bladder by the balloon, a movement of the skin in the lateral and dorsal parts of the thorax was observed. It was at first supposed that this might be a manifestation of the visceromotor reflex. However, further analysis on 34 cats showed that the reaction still persists after the hair has been removed by the cutting or shaving, and also persists after the intercostal nerves which supply the skin with vegetative fibers are severed. In a shaved animal one may readily observe that the skin moves on the underlying tissues when the gall bladder is stimulated. This movement is mainly in a longitudinal direction craniad, with a transverse dorsad component which is most marked in the region immediately below the shoulder. It is bilateral and often more distinct on the right side than on the left.

If the skin is separated carefully from the underlying tissues, after a median incision on the sternum, it is easily seen that this reaction is due to a contraction of the thin sheet of muscle lying immediately under the skin, which is known as the panniculus carnosus. This contraction of the panniculus carnosus may be observed as well when the fibers are adhering only to the skin as when they are still in contact with the underlying trunk muscles. Since we are dealing with a visceral reflex which has for its

effector the panniculus carnosus, we have named it the viscero-pannicular reflex.

If the animal is in good condition, the contraction of the pannicular muscle may be maintained as long as the visceral stimulation is continued, and it has the character of a tonic or mixed tonic-clonic reaction (figs. 1 and 2). This is shown by records obtained by sewing a string into the

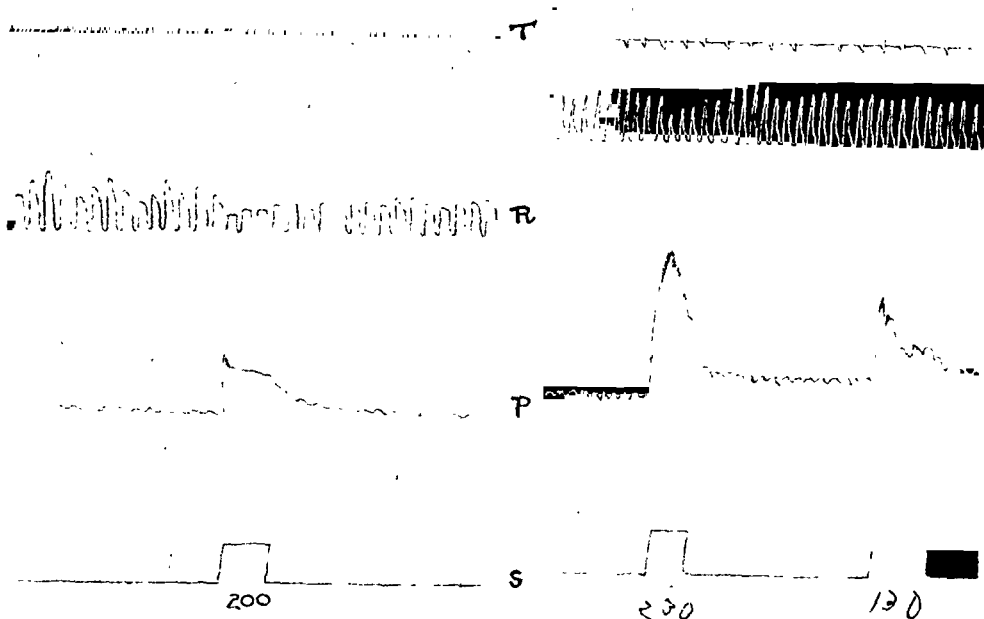


Fig. 1

Fig. 2

Fig. 1. Decerebrate cat. *P*, contraction of the panniculus carnosus; *R*, respiration (tambour connected with tracheal cannula); *S*, stimulation (pressure on balloon in gall bladder in millimeters mercury); *T*, time in seconds.

Fig. 2. Decerebrate cat. *P*, contraction of the panniculus carnosus; *R*, respiration; *S*, stimulation; *T*, time in 5 second intervals.

First stimulation (pressure 230 mm. Hg) elicits contraction of the panniculus carnosus and inhibition of respiration; 2nd stimulation (130 mm. Hg) produces weaker contraction of the panniculus carnosus, but no change in respiration.

skin of the dorsal part of the thorax, and connecting this with a muscle lever. In some records, the skin was intact, while in others it was separated from the underlying tissues. Simultaneous records of respiration showed that the viscero-pannicular reflex is often present when visceral stimulation fails to elicit a change in respiration (fig. 2). The maximum pressure employed in stimulation of the gall bladder was 350 mm. mercury, while the minimum effective pressure was usually 80 mm. mercury, al-

though a lower pressure (15 mm. mercury) was occasionally sufficient to evoke a response.¹

A similar but weaker response was obtained by distention of the duodenum, while dilatation of the urinary bladder failed to give a definite reaction.

When the right splanchnic nerve is severed in the abdomen or in the thorax, stimulation of the gall bladder no longer evokes a response. This is in agreement with the statements of Schrager and Ivy (1928) and of Davis, Pollock and Stone (1932) who found, in a study of the respiratory reaction to dilatation of the gall bladder and the biliary ducts, that these reactions are abolished by cutting the right splanchnic nerve.

Faradic stimulation of the central end of the right splanchnic nerve elicits a distinct contraction of the panniculus carnosus even before decerebration, but after decerebration the reaction is increased and can be elicited with weaker stimuli than before decerebration. On faradic stimulation of the left splanchnic nerve, with the brain intact and under superficial ether anesthesia, a slight movement of the skin of the homolateral side can be obtained. This reaction, however, is much more marked after decerebration.

These observations indicate that the centripetal part of the visceropannicular reflex arc is by way of the splanchnic nerve.

The central part of the reflex arc is located entirely within the spinal cord. The higher parts of the central nervous system are not necessary for the maintenance of this reflex, since it is still present after transverse section between the medulla oblongata and the spinal cord. The centrifugal part of the reflex arc is by way of the eighth cervical and first thoracic nerves, and then by the anterior thoracic nerves, since severing these nerves abolishes the reflex. This is in agreement with the statement of Langworthy (1924) that the panniculus carnosus in the cat is supplied by the nervi thoracales anteriores.

SUMMARY

In decerebrate cats, distention of the gall bladder or duodenum or faradic stimulation of the splanchnic nerves evokes a contraction of the panniculus carnosus muscle (viscero-pannicular reflex) which results in a movement of

¹ These variations in the minimum amount of pressure required to evoke a response on stimulation of the gall bladder may be related to the great variability which was observed in the size and other characteristics of the gall bladder. It must also be noted that the elasticity of the balloon plays a part in determining the minimum effective stimulating pressure, since a part of the stimulating pressure is always employed in overcoming this elasticity.

the skin of the trunk on the underlying tissues. This reaction is a spinal cord reflex, with its afferent pathway through the splanchnic nerve and its centrifugal pathway through the nervi thoracales anteriores.

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THE SUBSTANCE IN HUMAN SEMINAL FLUID AFFECTING UTERINE MUSCLE¹

JESSIE REED COCKRILL, EDGAR G. MILLER, JR. AND
RAPHAEL KURZROK

*From the Departments of Biological Chemistry and Obstetrics and Gynecology, College
of Physicians and Surgeons, Columbia University, and Sloane Hospital,
New York*

Received for publication April 25, 1935

It was reported by Kurzrok and Lieb (1) that human semen affected human uterine muscle. Normally, the effect was relaxation of the muscle. In certain cases (depending apparently on differences in the semen or in the uterus or in both) the effect was contraction. These effects have been demonstrated both *in vitro* and *in vivo*. The clinical data in the cases studied have shown the probability of a relation of this phenomenon to certain aspects of the problem of sterility.

The present studies were undertaken to identify the substance, in semen, responsible for this pharmacologic action.

Muscle strips from more than 400 human uteri obtained at operations in Sloane Hospital, and more than 200 specimens of seminal fluid, obtained from 75 patients in the Vanderbilt Clinic and private patients, were used. The semen samples from 65 of these patients caused relaxation of uterine muscle (fig. 1). Each sample was tested on strips from different uteri. In these relaxing cases it was found that, while the semen relaxed most uteri, at least one uterus would respond by contraction. Samples from 10 patients of the series were, on the contrary, found always to cause contraction (figs. 2 and 3).

The tests were done by the usual kymograph technique. The muscle strips were approximately 1 cm. in length and 1 gram in weight, and were suspended in 100 cc. of oxygenated Ringer solution at 37.5°C. The amount of seminal fluid added to this was usually 0.4 cc. or its equivalent.

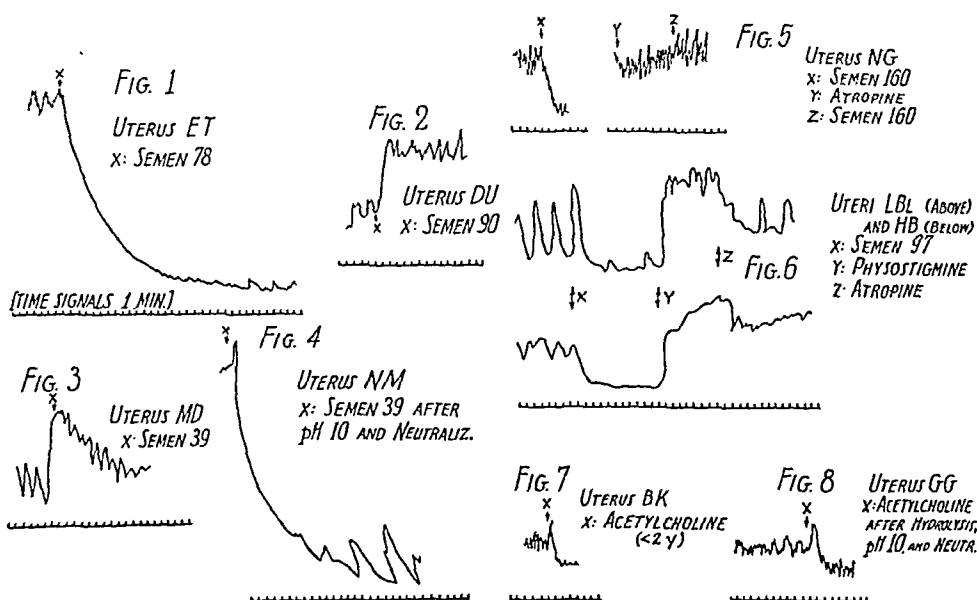
The contracting substance was first studied. Its activity slowly diminished with time at room temperature. Contracting semen, brought to pH 9.5 to 10 by addition of 0.4 N sodium hydroxide, allowed to stand for 30 minutes, and then neutralized, caused relaxation of uterine muscle (figs. 3 and 4). Similarly treated at pH 11 or above, it became inactive. Boiling the semen destroyed its activity. The active substance was found

¹ This work was aided by a grant from E. R. Squibb & Sons.

to be dialyzable through parchment or collodion membranes. It was soluble in water or in 95 per cent alcohol. An active fraction, prepared by drying, alcohol extraction, evaporation, and washing with dry ether, gave a negative biuret reaction.

Physostigmine, which itself had no pharmacologic effect on the muscle, enhanced the contracting action of the semen. Atropine abolished the action without altering the normal rhythm of the muscle.

Contracting semen which had been inactivated by boiling was treated with acetyl chloride. This gave a restored and enhanced contracting action. Esterase, prepared from pig liver, was shown to greatly diminish the activity. We suggest that our inability to abolish it completely



with esterase may have been due to the fact that the high level of pH necessary for complete hydrolysis could not be maintained on account of its effect *per se* on the active substance.

These results indicate that the contracting substance is acetyl choline or some very similar material. The pharmacologic data can be duplicated in detail with human uterine muscle, using pure acetyl choline. The amount present in semen is too small for chemical isolation or determination, but comparative dosage data suggest that the concentration in a uterus-contracting semen is of the order of about 0.01 mgm. in 1 cc.

Our experiments with the seminal samples causing relaxation of uterine muscle have led us to the tentative conclusion that the relaxing action is also due to acetyl choline in smaller dosage.

It appears that all semen specimens contain some of the contracting substance (acetyl choline), since normal relaxing samples could be shown

to cause contraction of some uteri. Also, when a specimen which relaxed most uteri was dried and the alcohol-soluble fraction from it concentrated and used in larger doses, it then caused contraction. Uteri of other animals (guinea pig, rabbit) which are extremely sensitive to acetyl choline, were invariably contracted by human semen. Relaxing semen gave an acetyl choline action on rabbit intestine.

The relaxing substance had the same solubilities as the contracting. It was heat labile, slowly decreased with time in water solution, and was destroyed by treatment with sodium hydroxide (pH 11) for 30 minutes. Esterase definitely lessened the activity.

Atropine (in amounts which alone had no effect on the muscle) abolished the relaxing effect of the semen (fig. 5). Addition of physostigmine to a relaxing semen invariably reversed the action, producing a contraction (fig. 6). This contraction was abolished by atropine.

Relaxing semen (1 cc.) caused a fall in the blood pressure of a pithed cat comparable to 0.001 mgm. of acetyl choline.²

Efforts to produce relaxation of uterine muscle with pure acetyl choline in exceedingly small doses (a few gammas) were several times successful (fig. 7). Dilute aqueous solutions of acetyl choline, treated with sodium hydroxide (pH 10) for 10 minutes, and then neutralized, caused definite relaxation (fig. 8).

Another property of semen and of acetyl choline was noted. Many uterine strips which were completely inert when suspended in the Ringer solution, giving a straight line record on the kymograph paper, promptly assumed a normal activity with normal rhythm after treatment with these materials; also, there was frequently a definite improvement in the rhythm and tone of active strips.

These facts, together with the apparent relation of uterine relaxation or contraction to the acceptance or expulsion of the sperm, suggest an important physiologic rôle for acetyl choline in semen.

In our series there were a few interesting variants from the general results. Occasionally a semen was found to abolish the normal rhythm of the muscle, but not to alter the tone. This type, retested on the same muscle after alkali treatment (pH 10) and neutralization, gave marked relaxation. These results were considered to be a function of the amount of acetyl choline present. Several semen samples produced no effect when tested on many different uteri. These specimens were definitely abnormal in showing only a few, non-motile, sperm. Other specimens showing the same morphological abnormality gave normal pharmacologic effects.

The factors conditioning the responses of uterine muscle to the action of acetyl choline and other substances are being investigated.

² This test was made by Doctor Mulinos, of the Department of Pharmacology.

We are indebted to Professor Watson for making available the clinical facilities of Sloane Hospital and Vanderbilt Clinic, and to Professors Lieb and Mulinos, of the Department of Pharmacology, for help and advice.

SUMMARY

Acetyl choline or some similar substance is present in human seminal fluid. When present in unduly large amounts it causes contraction of human uterine muscle. It is probably also responsible for the normal relaxation of uterine muscle. Its pharmacologic activity is of importance in the physiology of sperm entry into the uterus.

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A COMPARATIVE STUDY OF SODIUM, CHLORIDE AND BLOOD PRESSURE CHANGES INDUCED BY ADRENAL INSUFFICIENCY, TRAUMA AND INTRAPERITONEAL ADMINISTRATION OF GLUCOSE^{1,2}

W. M. PARKINS, A. R. TAYLOR AND W. W. SWINGLE

From the Biological Laboratory, Princeton University

Received for publication May 1, 1935

Several investigators have recently emphasized the importance of certain specific fluid and electrolyte changes associated with adrenal insufficiency (Swingle et al., 1933-34a, b, c, d; Loeb et al., 1933a, b; Harrop et al., 1933a, b, c; Zwemer and Sullivan, 1934). Sodium, fluid and circulatory changes analogous to those characteristic of adrenal insufficiency have also been shown to follow intraperitoneal injections of large quantities of glucose (Gilman, 1934).

Many of the signs and symptoms of secondary shock are strikingly similar to those induced by adrenal insufficiency and also by intraperitoneal glucose injections. It seemed important, therefore, to determine whether serum sodium, chloride and arterial pressure changes in traumatic shock might not also be comparable, and to what extent certain phenomena following trauma and glucose administration were influenced by the presence or absence of adequate cortical hormone.

The type of adrenalectomized dog used for experimental purposes in this laboratory has been previously described (Swingle et al., 1933-34). All dogs employed were in normal health and nutritive condition, and the adrenalectomized animals had been operated for periods varying from two months to one year previous to use in these experiments. The maintenance dose of adrenal cortical hormone for the adrenalectomized animals was 0.1 to 0.2 cc. per kgm. per day unless otherwise stated. The dosage used for reviving the animals from shock and collapse was 3 cc. per kgm. given daily in divided doses until the blood pressure and electrolyte pattern had returned to normal. Mean blood pressure was determined by direct needle puncture into the femoral artery (Parkins, 1934). Serum sodium was determined by the method of Butler and Tuthill (1931) and

¹ These investigations have been aided by a grant from The Rockefeller Foundation.

² We are indebted to Parke Davis & Co. for considerable quantities of fresh beef adrenals used in preparation of cortical hormone.

chlorides by that of Van Slyke and Sendroy (1923) as modified by Eisenman (1929). All blood samples were taken from the femoral artery, and were drawn, delivered and centrifuged under oil.

Deep ether anesthesia was used on both normal and adrenalectomized animals subjected to trauma and care taken that the dogs did not suffer pain during the experimental procedure. Muscle trauma to the posterior extremities was produced by the method described in a previous study (Swingle and Parkins, 1935).

I. *A comparison of the serum sodium and chloride changes of adrenal insufficiency with those following trauma to the extremities of the healthy, vigorous adrenalectomized and the unoperated animal.* The serum sodium, chloride and arterial pressure have been followed in 6 adrenalectomized and 5 normal dogs subjected to muscle trauma and have been compared with the electrolyte and arterial pressure changes occurring in a typical case of uncomplicated adrenal insufficiency and recovery on extract treatment. Table 1 gives the pertinent data in representative cases of 1, adrenal insufficiency and recovery; 2, trauma to an adrenalectomized dog and the effects of treatment of the resulting shock with cortical hormone, and 3, the typical electrolyte changes observed in the intact dog following severe trauma.

The magnitude of the serum sodium and chloride decline in adrenal insufficiency observed by us (table 1 A) compares favorably with that reported by Loeb et al. (1933b) and Harrop et al. (1933c) for this species. It is of interest to note that the average decrease of 14.8 M.-eq. of sodium and 11 M.-eq. of chloride which occurs within a period of 6 to 12 hours following trauma to the adrenalectomized dog, maintained in normal health and vigor by adequate injections of cortical hormone, is of the same magnitude (table 1 B) as in adrenal insufficiency following withdrawal of hormone (table 1 A). In the one case (trauma) the sodium fell within a few hours, in the other case (insufficiency) a similar decline required a period of 11 days (dog 35, table 1).

The blood samples were taken from the adrenal insufficient and shocked animals when the arterial pressure had declined to approximately 50 mm. of Hg. At this blood pressure level the general symptoms of secondary (traumatic) shock were quite obvious. Cortical hormone (3 cc. per kgm.) was injected intravenously. One shocked dog from which hormone was purposely withheld died one hour after the blood sample was taken. In all other cases, following hormone injection, the blood pressure slowly began to rise, and the shock symptoms disappeared. A normal level of serum sodium and chloride, blood pressure and pulse rate was reestablished in a manner similar to that occurring in recovery from uncomplicated adrenal insufficiency (table 1; compare animals 35 and 3).

The normal dog with adrenal glands intact, even though traumatized to a

far greater degree than the equally active and vigorous adrenalectomized animal, nevertheless reveals a comparatively negligible decline in serum sodium (average 5.5 M.-eq.) and chloride (average 3.8 M.-eq.), when in profound shock and collapse. (Table 1. Compare dogs 3 and 10.) It should perhaps be emphasized that previous to traumatization, these unoperated dogs were in no better physiological condition, at any rate so far

TABLE 1

Sodium, chloride, and blood-pressure changes in adrenal insufficiency and trauma

DATE	TIME	BLOOD* PRES- SURE	PULSE	SERUM SODIUM	SERUM CHLO- RIDE	REMARKS
A. Adrenal insufficiency. Dog 35 ♂						
		<i>m.m.Hg</i>	<i>per min.</i>	<i>m.-eq.</i>	<i>m.-eq.</i>	
Jan. 23	a.m.	104	76	145.0	107.2	Extract discontinued
Jan. 29	a.m.	75	120	129.6	97.8	Normal food intake, active
Feb. 4	a.m.	50	60	123.4	97.8	Extract injected. Weak, some- what spastic
Feb. 8	a.m.	98	68	136.9	107.6	Normal food intake, active and vigorous
Feb. 10	a.m.	102	72	144.6	108.1	Maintenance extract
B. Trauma—Adrenalectomized. Dog 3 ♂						
Dec. 17	10 a.m.	103	96	141.7	107.8	Healthy, active, vigorous
Dec. 17	5 p.m.	62	184			Slight weakness, inactive
Dec. 17	10 p.m.	48	180	125.2	96.8	Shock, weak, spastic, extract in- jected
Dec. 20	10 a.m.	108	104	135.8	105.6	Appears practically normal, eats full ration
Dec. 23	11 a.m.	106	92	140.1	105.8	Normal, maintenance extract
C. Trauma—Unoperated. Dog 10 ♂						
Nov. 16	11 a.m.	110	92	145.9	108.6	Normal
Nov. 16	6 p.m.	66	212			Mild shock, weak, inactive
Nov. 16	11 p.m.	30	160	141.2	103.0	Deep shock, prostrated

* All dogs were carefully trained for blood-pressure determinations unless otherwise stated.

as activity, vigor, appetite, weight and general health are concerned, than the adrenalectomized dogs on maintenance doses of hormone. Nevertheless, the former obviously possessed some factor which enabled them to maintain a nearly normal serum sodium and chloride concentration in the face of a very severe and extensive trauma, which even in mild degree, produced a critical diminution of these electrolytes in the healthy adrenalectomized dog (table 1 B). This factor, present in the intact, but lacking

in the adrenalectomized animal, we consider to be an adequate reserve of cortical hormone in the glands of the unoperated dog. That such is the case is strongly indicated by the fact, amply demonstrated previously (Swingle and Parkins, 1935) and repeated here in connection with electrolyte studies, that the healthy, vigorous, adrenalectomized dog thrown into profound shock and collapse by mild trauma to one extremity, can easily be restored to normal health by adequate injections of cortical hormone (table 1 B).

Histological examination of the adrenals from dogs which had succumbed to muscle trauma, revealed marked diminution of the cortical lipoid, and hemorrhagic changes indicative of functional stress and strain on the glands (Donahue and Parkins, in press).

Despite the marked differences in serum sodium and chloride concentration revealed by the two types of shocked dogs, the diminution in arterial pressure and the gross symptoms of secondary shock were very much the same in both the traumatized normal and adrenalectomized animals.

In the shocked adrenalectomized animals, the low arterial pressure, hemoconcentration and death are associated primarily with a drastic reduction of the serum sodium and chloride, a generalized shift of fluid and certain electrolytes from the blood stream to the tissues and interstitial tissue spaces, and failure of the blood diluting mechanism. The single traumatized limb was invariably only moderately swollen, as contrasted to the greatly swollen and distended tissue in both posterior extremities of the shocked normal dog. The local loss of fluid into the area of injury following the mild degree of trauma necessary to induce profound shock and collapse in the adrenalectomized dog is apparently a minor factor in blood volume reduction. In the normal dog with adrenals intact this local loss of fluid and whole blood into the severely injured limbs is sufficient to reduce the blood volume to the death level.

Since the traumatized adrenalectomized animals are almost invariably anuric, or practically so, throughout the experimental period, it is obvious that wastage of fluid and salts by way of the kidney is not an important factor in the blood volume and electrolyte reduction.

In the shocked animal with intact adrenals the electrolyte changes are relatively slight as compared to those occurring under similar condition in the adrenalectomized dog. The diluting mechanism remains essentially normal for long periods, as indicated by the prompt blood dilution which follows fluid ingestion by mouth in such animals, contrasted with the shocked adrenalectomized animal where such fluid intake is of no avail.

The investigations of Blalock (1930), Parsons and Phemister (1930), Freedlander and Lenhart (1932) and others, clearly demonstrate that the secondary shock following trauma to muscle masses in normal dogs is associated with a local loss of plasma and whole blood into the injured area,

equivalent to approximately one-half of the total blood volume. These investigators emphasize that this loss is of itself sufficient to cause the low blood pressure and subsequent death of the animal. Observations made in this laboratory on a large series of shocked normal dogs agree with this viewpoint.

II. *Serum sodium, chloride and blood pressure changes resulting from shock following a single stage bilateral adrenalectomy.* The shock syndrome which follows bilateral extirpation of the adrenals at a single stage operation has been adequately described in an earlier communication (Swingle and Parkins, 1935). We are concerned here only with the serum sodium and chloride changes associated with this condition.

TABLE 2

Sodium, chloride, and blood-pressure changes following single stage bilateral adrenalectomy

DOG	DATE	TIME	BLOOD PRES- SURE	PULSE	SERUM SODIUM	SERUM CHLO- RIDE	REMARKS
			<i>m.m.Hg</i>	<i>per min.</i>	<i>m.-eq.</i>	<i>m.-eq.</i>	
14	Jan. 8	9:30 a.m.	116	104	143.9	113.2	Normal, untrained
	Jan. 8	11:40 a.m.	132*	172			10 minutes after 40-minute period for complete op.
	Jan. 8	8:05 p.m.	67	140			Weak, lethargic
	Jan. 9	12:30 a.m.	34	124	133.8	100.2	Prostrate, died 10 minutes later
16	Feb. 3	10:00 p.m.	104	80	145.9	111.0	Normal
	Feb. 4	8:00 a.m.	72	204			Lethargic
	Feb. 4	9:40 p.m.	53	200			Spastic
	Feb. 4	11:35 p.m.	38	160	129.8	102.5	Severe shock, died within 2 hours

Dog 14 was untrained for blood-pressure determination.

* Under nembutal anesthesia.

Table 2, dogs 14 and 16, illustrate representative data obtained from five such experiments. The two cases listed show values nearest the mean. The per cent decrease in both sodium and chloride depended largely upon the severity of the symptoms and blood-pressure level at the time the sample was taken. It will be noted that these electrolytes declined considerably. The blood changes and gross symptoms observed in this type of experimental animal were somewhat similar to those following trauma to an extremity of the adrenalectomized dog. (Compare table 2 and table 1 A and 1 B.)

III. *The effect of intraperitoneal glucose injections upon the serum sodium, chloride and arterial pressure of adrenalectomized and unoperated dogs.* Gilman (1934) demonstrated that when isotonic glucose is injected intraperi-

toneally and later followed by paracentesis, a marked fall in serum sodium and chloride occurs within a few hours. The arterial pressure declines owing to the hemoconcentration induced by the internal fluid and electrolyte shift. Animals so injected were also shown to be extremely sensitive to slight hemorrhage and to exhibit other changes analogous to adrenal insufficiency.

In view of the theory of adrenal cortical function adopted by workers in this laboratory (Swingle et al., 1933a, 1934b, c, d), it seemed probable that the resistance of an animal to the electrolyte and fluid shift induced by intraperitoneal glucose administration would depend on the quantity of cortical hormone available, either from the intact adrenals or as supplied by extract injections. It was with this idea in mind that the following experiments were performed on the unanesthetized, intact and adrenalectomized dog.

Rapid intraperitoneal injections of 50 cc. and 100 cc. of 5.5 per cent glucose per kgm. were made through a 12 gauge needle attached by gum tubing to a pressure flask. A local anesthetic was used at the site of a small incision for paracentesis. The rapid removal of an equal volume of fluid by paracentesis was facilitated by use of an aeration tube appropriately connected through a receiving flask to a suction pump.

The pertinent data representing experiments on 8 normal and 8 adrenalectomized dogs are illustrated in table 3. A representative case of the adrenalectomized series (dog 25) collapsed within one hour after the injection of 100 cc. of glucose per kgm. of body weight. The blood pressure was at shock level and the decrease in serum sodium and chloride was comparable to that which occurs over a period of three hours in the representative case of the unoperated series (dog 24) and also in true adrenal insufficiency (table 1 A). It was necessary to withdraw the fluid by paracentesis and inject cortical hormone (3 cc. per kgm.) intravenously to save the adrenalectomized animals. The normal dog, on the other hand, was not so seriously affected, as shown by the 30 mm. decrease in pressure, and the general symptoms (table 3 A) when contrasted to a 60 mm. change and collapse by the adrenalectomized dog (table 3 B). The animals with intact adrenals, although somewhat weakened and lethargic as a result of the hemoconcentration induced by the electrolyte and fluid shift resulting from the glucose injections, were able to withstand a moderate amount of muscle trauma after paracentesis. All recovered from the glucose alone and many from both treatments without hormone injection.

With the injection of 50 cc. of glucose per kgm. the adrenalectomized dog (table 3 D) showed at the end of the 3 hour period sodium and chloride changes comparable to those in the unoperated dog 24 (100 cc. per kgm. and approximately double those produced in the unoperated dog 34 (50 cc. per kgm.). It was unnecessary to withdraw this quantity of fluid from

TABLE 3

*Sodium, chloride, and blood-pressure changes in normal and adrenalectomized dogs following intraperitoneal injection of isotonic glucose**

DATE	TIME	BLOOD PRES- SURE	PULSE	SERUM SO- DIUM	SERUM CHLO- RIDE	SYMPTOMS	REMARKS
A. Unoperated. Dog 24 ♂—9.9 kgm. (100 cc. per kilo)							
	hours	mm. Hg	per min.	m.-eq.	m.-eq.		
Jan. 2	11:00 a.m.	112	96	141.0	110.0	Normal	Glucose injection
Jan. 2	2:00 p.m.	82	180	124.4	92.0	Depressed, restless	Fluid withdrawn
Jan. 7	10:00 a.m.	110	88	142.6	108.4	Normal	Recovery, paracentesis trauma
B. Adrenalectomized. Dog 25 ♂—11.7 kgm. (100 cc. per kilo)							
Nov. 30	10:45 a.m.	102	80	139.4		Healthy, active	Min. maint. extract
Nov. 30	11:50 a.m.	42	176	125.6		Collapsed	Fluid withdrawn—Ex- tract
Dec. 6	10:30 a.m.	104	72	139.8		Normal	Healthy, active, vigor- ous appetite
C. Unoperated. Dog 34 ♀—9.4 kgm. (50 cc. per kilo)							
Feb. 4	11:45 a.m.	124	120	147.2	113.8	Normal	Untrained for B.P.
Feb. 4	3:00 p.m.	112	108	139.0	106.8	Normal	Vigor, appetite, unaf- fected
Feb. 6	2:00 p.m.	116	104	146.8	115.6	None ex- ternally	Unchanged
D. Adrenalectomized. Dog 30 ♂—13.0 kgm. (50 cc. per kilo)							
Feb. 2	11:10 a.m.	104	80	142.2	107.8	Normal	Maint. dose hormone
Feb. 2	2:15 p.m.	50	124	125.1	98.7	Weak, spastic	Extract intravenously
Feb. 7	10:00 a.m.	106	72	140.7	106.4	Active vig- orous	Excellent condition
E. Adrenalectomized. Dog 32 ♂—12.5 kgm. (50 cc. per kilo)							
Nov. 19	10:30 a.m.	82	124	144.3		Eats, ac- tive	Submin. maint. hor- mone
Nov. 19	11:30 a.m.	34	102			Complete collapse	No extract injected
Nov. 19	12:40 a.m.	28	92	118.3		Prostrated	Death within 10 minutes

* This table illustrates representative cases from sixteen experiments.

the adrenalectomized animals when adequate hormone was injected intravenously.

The blood sugar elevation (approximately 50 to 100 per cent) induced in the adrenalectomized dog by intraperitoneal glucose injection is quite inadequate to produce any of the injurious effects observed. The deleterious effects resulting from these rapid shifts in fluid and electrolytes and consequent lowering of blood volume and arterial pressure in the healthy adrenalectomized animals (table 3, B and D) are just as drastic as similar changes in uncomplicated adrenal insufficiency (table 1 A). The response to cortical hormone injection is likewise the same in both cases.

An interesting case is that of dog 32 (table 3 E). This animal was given a subminimal maintenance dose of cortical hormone for a few days and this was reflected by a decrease in blood pressure, and an increase in pulse rate and urea nitrogen at the time he was used in the experiment. However, from external observations, he appeared to be normal at the time of injection of 50 cc. of glucose per kgm. In less than one hour he was prostrate and died when a blood sample was drawn at the end of a two hour and ten minute period. The serum sodium level was reduced somewhat below that observed in an animal receiving adequate maintenance hormone, subjected to the same quantity of glucose over a period of three hours (table 3 D).

It is obvious from these experiments that when the serum sodium and chloride concentrations of an adrenalectomized dog are lowered from normal levels, either by glucose injection or trauma, to levels as low as those in true adrenal insufficiency, hemoconcentration and circulatory failure result. Blood dilution does not occur in the absence of cortical hormone, even when the serum sodium and chloride concentrations are only moderately diminished. Following hormone injection, progressive compensation by blood dilution occurs while the serum sodium and chloride levels may have risen only slightly above the lowest levels. A normal blood pressure is invariably attained before the normal electrolyte pattern is established.

Silvette and Britton (1935) report that in the normal cat the tissue and serum sodium and chloride can be reduced to levels much lower than those observed in adrenal insufficiency without deleterious effects.

By injecting a large quantity of glucose intraperitoneally (approximately 100 per cent more than that required for an equal M.-eq. electrolyte reduction in the healthy, vigorous, adrenalectomized dog), the serum sodium and chloride may be lowered in the animal with intact adrenals to levels similar to those of adrenal insufficiency. Nevertheless, the circulation is not so seriously impaired as evidenced by the arterial pressure and general symptoms. These animals spontaneously recover. The

question arises as to why there should be such a marked difference in these respects between the intact and the adrenalectomized dog when the serum sodium and chloride levels have been reduced to similarly low levels. The only important variable between the two types of animal is the presence of adrenal cortical tissue in the intact dog. In our opinion, the presence of such tissue is the answer to this question, for when the hormone is present in adequate amounts blood dilution occurs even at extremely low serum sodium and chloride concentration. This fact can be easily demonstrated by administering cortical hormone to adrenalectomized dogs: 1, in collapse from traumatic shock; 2, verging on death from adrenal insufficiency, and 3, prostrate as a result of intraperitoneal glucose injections. The adrenalectomized dog subjected to such treatment succumbs unless injected with hormone.

SUMMARY AND CONCLUSIONS

1. Mild muscle trauma to healthy vigorous adrenalectomized dogs induces a rapid (6 to 12 hr.) decrease in serum sodium and chloride to concentrations similar to those characteristic of uncomplicated adrenal insufficiency (approximately 6 to 12 days after withholding hormone). The circulatory collapse, as evidenced by blood pressure and symptoms, associated with these electrolyte and fluid changes are likewise comparable.

2. The normal dog subjected to severe trauma to both hind legs shows only a slight reduction in serum sodium and chloride even when in extreme secondary shock. The circulatory failure is due primarily to low blood volume induced by extensive loss of whole blood and plasma into the severely injured areas.

3. The adrenalectomized dog loses a negligible amount of fluid into the slightly injured tissue. The rapid hemoconcentration, low blood pressure and death are due to a generalized shift of fluid and electrolytes from blood stream to the tissues and interstitial tissue spaces, and failure of the diluting mechanism owing to absence of the adrenal cortex. Following cortical hormone injection the normal internal electrolyte and fluid balance is reestablished.

4. A decrease in serum sodium and chloride is associated with shock resulting from the single stage bilateral extirpation of the adrenals in this species.

5. The adrenalectomized dog although in equally good physiological condition is far more susceptible to the decrease in serum sodium and chloride and associated disturbance in fluid balance following intraperitoneal injections of isotonic glucose than is the animal with intact adrenals.

6. When these serum electrolytes are reduced by glucose in the adrenalectomized dog to levels similar to those of uncomplicated adrenal insufficiency the circulatory phenomena and symptoms are comparable in both

cases. The adrenalectomized dog invariably succumbs to such abnormal electrolyte and fluid shifts induced by glucose administration unless large doses of cortical hormone are injected.

7. The quantity of glucose must be approximately doubled to produce an equivalent diminution in serum sodium and chloride in the animal with intact adrenals. Despite the electrolyte and fluid disturbances, the circulatory changes and symptoms are not nearly so severe as those occurring in the adrenalectomized dog lacking hormone, and spontaneous recovery is invariable.

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A COMPARISON OF THE INFLUENCE OF FASTING UPON THE TOLERANCE TO GLUCOSE AND GALACTOSE

EATON M. MacKAY, H. C. BERGMAN AND RICHARD H. BARNES

From The Scripps Metabolic Clinic, La Jolla, California

Received for publication April 17, 1935

There has been an accumulation of evidence which indicates that the metabolism of galactose may be considerably different from that of glucose. The ketolytic and nitrogen sparing action of galactose in fasting humans and in those receiving a ketogenic diet was observed by Deuel, Gulick and Butts (1) to be superior to that of glucose. Galactose is a relatively poor glycogen former when fed under the same outward conditions as glucose (2) probably because, at least in rats and dogs, it is lost so rapidly by the organism. However galactose formed glycogen is held much more tenaciously by the organism (3). Galactose unlike glucose is not effective in insulin shock (4) and cannot be used by liverless animals (5). Some investigators (4, 6) suggest the utilization of galactose by the diabetic organism but there is considerable evidence (7, 8) against this. Fasting, as is well known, reduces the tolerance for glucose in the normal subject (9, 10). There is suggestive evidence (11) that in the rabbit fasting is without any influence upon galactose tolerance. An investigation has been made of this point.

A typical result is presented here. Three adult male albino rabbits weighing 4.3, 4.4 and 4.3 kgm. respectively were given intravenous sugar tolerance tests to glucose and galactose, the curves for each sugar being determined both when they were fully fed and after a fasting period of 3 days. In every case the sugar was given in 50 per cent solution and the dose of sugar was always 9 grams. A preliminary blood specimen was taken and the sugar solution then given in the marginal ear vein. Four minutes were required for the injection and blood samples were taken 10, 30, 60, 90, 120, 180 and 240 minutes after the injection was completed. All blood specimens were taken by heart puncture and the proteins removed with Somogyi's (12) zinc hydroxide reagent. Somogyi's modification (13) of the Shaffer-Hartman method was used for the sugar determinations. The amount of sugar lost in the urine was determined in the galactose experiments. All of the results are expressed in terms of glucose.

The data presented in figure 1 demonstrate very clearly the influence of fasting upon the tolerance to glucose. With galactose on the other hand

there is no demonstrable influence of fasting. The blood sugar curves following galactose after full feeding and fasting are practically the same. This can hardly be due to differences in the amount of sugar lost in the urine. After feeding, an average of 2.84 grams of galactose was lost per rabbit and after fasting an average of 3.26 grams.

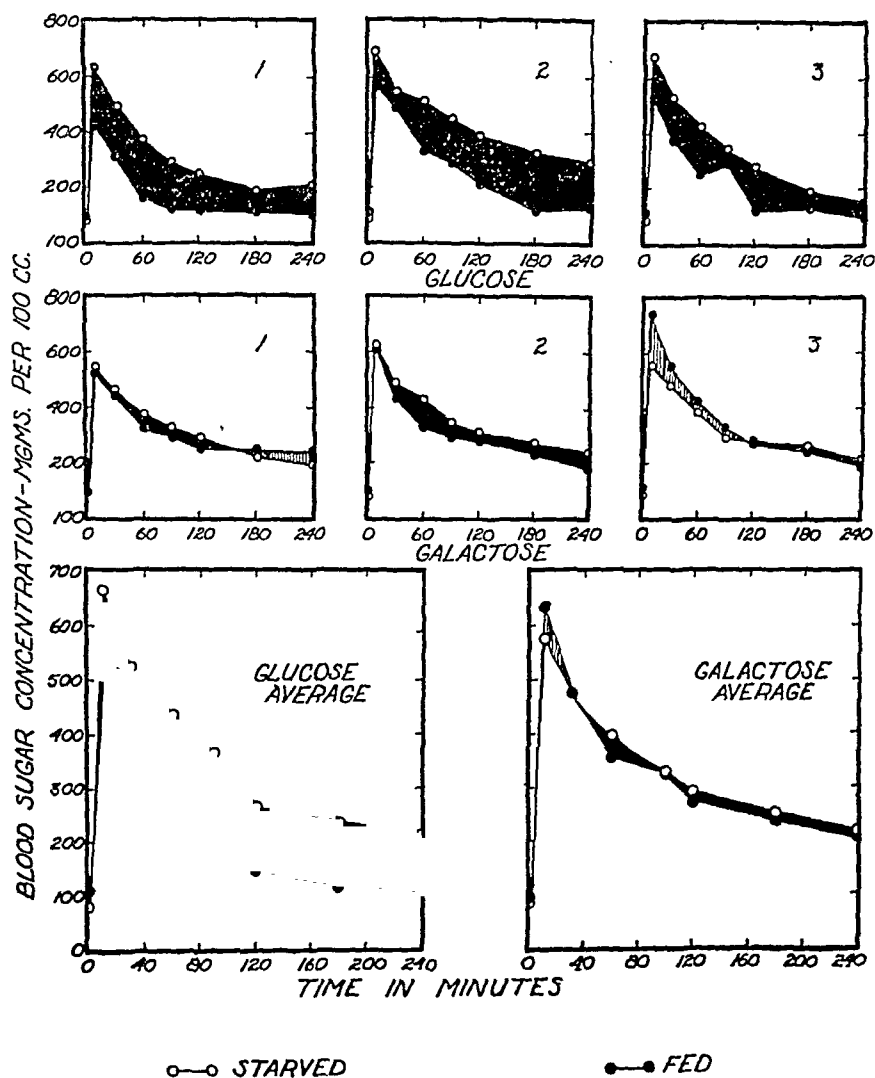


Fig. 1

It is generally accepted that the higher utilization rate or lower blood sugar curve following a second dose of glucose (14) has essentially the same basis as the higher sugar tolerance after feeding in comparison with fasting (9). With this view our findings are supported by the experiments of Sawada (15). He [found] that the intravenous injection of glucose in

rabbits was followed by a hypoglycemia after the hyperglycemia while after the injection of galactose the hypoglycemia phase was absent. A second injection of the same sugar 2 hours after the first injection was found to give a flatter blood-sugar curve in the case of glucose but not in that of galactose.

The experiments we have given an example of, and those of Sawada were carried out with intravenous galactose injections. The possibility of a difference in the results of intravenous and alimentary administration must be considered, particularly in view of the place of the liver (16) in determining the nature of the glucose tolerance curve. Then Nissen (17) has reported that young persons on a low carbohydrate diet had an abnormally high and protracted hyperglycemia following oral or intravenous adminis-

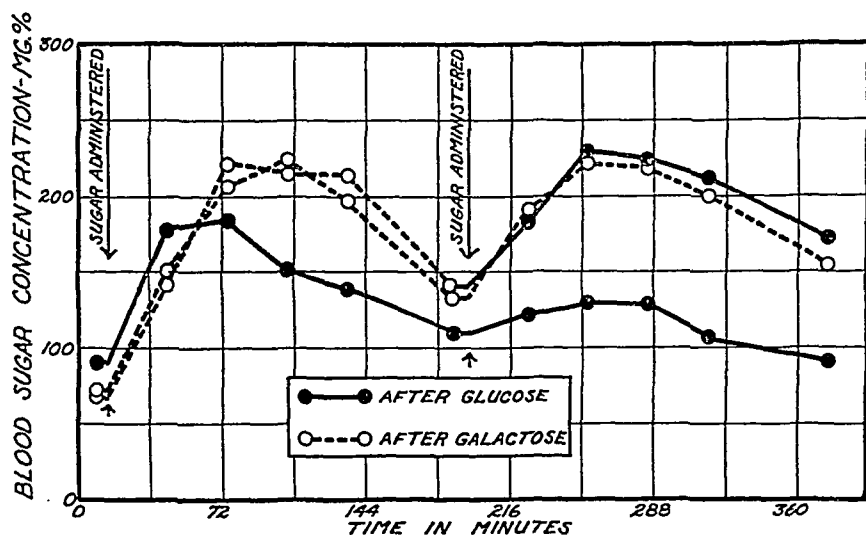


Fig. 2

tration of glucose with similar results following oral but not intravenous administration of galactose. The alimentary hyperglycemia from repeated doses of glucose and galactose was consequently examined.

Figure 2 presents a typical result. These curves were from 3 different male rabbits weighing 3.3, 3.3, and 3.2 kgm. respectively. They had been starved over night. A fasting blood sample was taken and then 3 grams per kilo of the desired sugar in 25 per cent solution administered by stomach tube. Three hours later a second dose of sugar was given. A second dose of glucose (after glucose) gave a very much flatter curve as might be expected (14). The preceding ingestion and absorption of galactose has no influence upon the hyperglycemia due to a second dose of this sugar. Glucose ingestion when preceded by galactose always gave curves similar to the fasting animal. These experiments confirm the results and conclusions drawn from the intravenous administration experiments.

SUMMARY

In contrast with that of glucose the blood sugar curve in the rabbit following intravenous galactose administration is not influenced by fasting.

Unlike that of glucose the alimentary hyperglycemia produced by galactose is uninfluenced by the previous ingestion of glucose nor is the blood sugar curve of a second dose of galactose essentially different from that of the first.

These findings are taken as additional evidence that there may be a fundamental difference in the metabolism of glucose and galactose.

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THE EFFECT OF AN ANTIDROMIC IMPULSE ON THE RESPONSE OF THE MOTONEURONE¹

R. LORENTE DE NÓ

From the Central Institute for the Deaf, St. Louis, Missouri

Received for publication May 3, 1935

Antidromic shocks have been recently used by the Oxford School (Denny Brown, 1928; Eccles, 1931; Eccles and Sherrington 1931; Eccles and Hoff, 1932) to analyse the process of excitation of the spinal motoneurons by stimuli delivered across a reflex arc. The interpretation of the experimental results was largely based upon the assumption that after arrival of the antidromic impulse the motoneurons enter into a period of refractoriness (absolute for no longer than 2.4σ and relative for no longer than 14σ ; Eccles, 1931). At the time the experiments were being carried out no other explanation seemed possible, because the axones were not known to be partially refractory for longer than 5σ (Eccles, 1931, p. 568). Later, however, evidence has been forthcoming which changes the theoretical basis upon which the Oxford School based the discussion of the experimental findings. On one hand the concept of recovery of nerve is in process of evolution (Gasser, 1935; Graham, 1933, 1935) and on the other hand the absolute refractoriness of the neurone, measured by more direct methods has been found to be no longer than that of the axon (Lorente de N6, 1935b).

It seemed therefore advisable to analyze the effect of antidromic impulses by means of a preparation in which the motoneurons receive stronger stimuli than those produced across an ordinary reflex arc.

TECHNIQUE. The preparation used (rabbit) has been described in a recent paper (Lorente de N6, 1935a). By means of stimulating electrodes placed on the floor of the fourth ventricle shocks were delivered to the pathways ending on the motoneurons of the third nerve and the action potentials developed by the internal rectus muscle were recorded. The shocks were produced by the double Thyatron stimulator (Schmitt and Schmitt, 1932), mentioned in a previous paper (Lorente de N6, 1935b).

Antidromic shocks produced by a similar stimulator were delivered to the oculomotor nerve in the base of the skull shortly after leaving the mid-brain. The diagrams in figures 1, 2 and 4 show the position of the electrodes

¹ The work reported in this paper has been aided by a grant from the Rockefeller Foundation.

and the paths for the stimuli in each experiment. For the purpose of the present study in which the normal rate of recovery of a nerve had to be determined, it was of paramount importance to keep the nerve as nearly as possible in a normal condition. Therefore the nerve was not prepared; shielded electrodes were introduced through the thalamus until their free points were obliquely placed across the oculomotor nerve. If only the effect of motor shocks had to be examined the third nucleus was destroyed, but if responses of the motoneurons had to be obtained the thalamus and midbrain were not disturbed. But at any rate, in no case was the blood supply of the nerve disturbed, nor of course was its temperature changed. These precautions perhaps account for the much longer recovery periods observed. But even with those precautions a deterioration of the nerve after prolonged stimulation was detected, which may be explained by polarization at the site of stimulation.

The internal rectus muscle was prepared with utmost care not to disturb its blood supply and was not exposed until by means of a suitable vaporizer the temperature of the surrounding air was raised to 37°C. The distal end of the muscle was connected with a silk thread to an isometric myograph and submitted to an initial tension no larger than 1 or 2 grams. The tension developed by the maximal twitch was found to remain constant within very narrow limits during the whole experiment (several hours).

The potentials were recorded with the amplifier and cathode ray oscillograph described in another paper (Blossom and Lorente de Nó, 1934). Two silver-silver chloride hooks applied on the muscle were used as recording electrodes. The ground electrode was placed on the belly of the muscle about 10 mm. from the end, the grid electrode on the killed end of the muscle.

RESULTS. *The recovery of the nerve muscle preparation.* This has been measured in terms of the height of the response to a second (testing) shock weaker than the first or conditioning shock. The height of the crest of the second response has been measured with fair accuracy by superposing the two films obtained with the conditioning shock alone and with both shocks and subtracting the former response from the latter.

The curves obtained by plotting height of conditioned response against interval between shocks (fig. 1) have a different significance when the shocks delivered to the nerve are maximal than when they are weak. If both shocks are maximal the shape of the curve is determined by the statistical distribution of the absolutely refractory periods of the nerve fibres and the size of the potential developed by the partially refractory muscle fibres. But if the second (testing) shock is weak the plotted curve is a fair indicator of the temporal course of the late recovery of the nerve fibres.

Curve 1 and 2 in figure 1 were obtained with supermaximal shocks, so

that the nerve fibres necessarily gave a second response as soon as they recovered from absolute refractoriness. The earliest second response of muscle was obtained at a stimulus interval of 0.52 or 0.60 σ . These values are indeed unexpectedly low; they are lower than those found in a previous

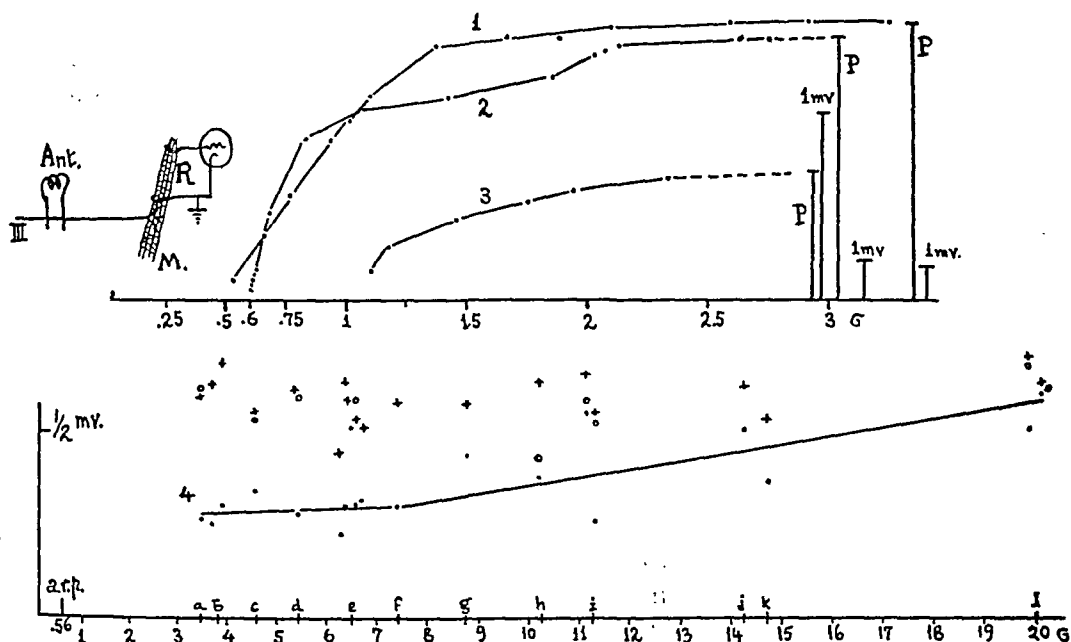


Fig. 1. Curves obtained by plotting the height of the crest of the second response of the nerve muscle preparation (ordinates) against the interval between stimuli in σ (abscissae). The stimulating electrodes (*Ant.*) were placed across the oculomotor nerve (III), the recording electrodes (*R*) on the internal rectus muscle (*M*). *P* is the potential developed by the response to the unconditioned second shock; scale (1 millivolt) at side.

1 and 2. Both shocks supermaximal (1, expt. 22/1/35; 2, expt. 17/1/35).

3. The second shock submaximal (expt. 20/11/34).

4. Both shocks very near threshold, but the second one slightly smaller (expt. 23/1/35). The points of the curve were obtained at the intervals marked by the letters *a* to *l*. No second response was observed at an interval of less than 3.5 σ . At each interval several films were taken, of both conditioned and unconditioned second response. The crosses mark the height of the response to the first shock, the circles the response to the unconditioned second shock and the dots the responses to the second shock preceded by the conditioning one. *a.r.p.*—absolute refractory period measured with two supermaximal shocks.

investigation (Lorente de N6, 1935b), because the technique has been improved and the muscle was kept in better condition; but they are still higher than the absolutely refractory period of the motor fibres themselves. Recent work of Gasser (1935) has shown that A nerve fibres may follow a stimulus frequency of 2000 shocks per second and therefore have to have

an absolutely refractory period less than 0.5σ . Therefore the new value (0.52 to 0.60σ) of the absolutely refractory period of the nerve muscle preparation does not change the classical belief that the muscle fibre, even when stimulated through the end plate, may not follow as high an impulse frequency as its axon, although it comes very close to it.

As already stated the shape of curves 1 and 2 in figure 1 can not be easily analyzed because it is determined both by the statistical distribution of absolutely refractory periods in the nerve and the recovery of the muscle action potential; nevertheless it can be concluded from those curves that the recovery of the muscle action potential is complete 2.5σ after the first response and therefore if a deficit in potential is found later, it has to be attributed to the failure of nerve fibres to respond to the second shock.

Thus, when the second shock is weakened the shape of the curve is primarily determined by the relative refractoriness of the nerve fibres. Curve 3 in figure 1 illustrates an experiment in which the testing shock produced an unconditioned response of about $\frac{1}{3}$ of the maximal motor twitch. The earliest second response was obtained at an interval between shocks no shorter than 1.2σ ; the recovery was complete at about 2.5σ .

Using still weaker testing shocks, which do not begin to be effective until 1.5 or 2σ after the conditioning one it has been customary to find depressed second responses until no less than 8 or 9σ after the first shock. Since at that stimulus interval the muscle fibres were developing full-sized potentials it had to be concluded that some nerve fibres failed to respond. But it has been also consistently found that the relatively refractory period thus measured diminished during the experiment, dropping nearly to the figure (5σ) mentioned by Eccles (1931).

Therefore it has been tried to determine the relatively refractory period at the very beginning of the experiment, without previous stimulation of the nerve. The result of a typical experiment is illustrated by curve 4 in figure 1. The shocks used were just above threshold and therefore stimulated only a few of the most irritable fibres. The crest of the action potential was variable in height within rather narrow limits, undoubtedly because the nerve fibres were showing the spontaneous changes in excitability mentioned by Blair and Erlanger (1933). Under such conditions the determination of the end of refractoriness had to be more or less statistical.

The experiment was carried out as follows: Beginning at an interval between stimuli of 8σ , at which the second response was still very much depressed, the testing shock was placed at progressively increasing times from the conditioning one, and at each distance several—at least 6—alternative determinations were made of the height of the unconditioned and of the conditioned second response; the positions of the crests were marked with dots on the face of the cathode ray oscillograph. Thus, two

series of groups of dots were obtained which did not coincide until the interval between shocks became as long as 20σ . Before that interval was reached the group of dots of the conditioned response were found consistently below those of the unconditioned one. Immediately afterwards the oscillograph was cleaned and several films were taken which have been used in the construction of curve 4 in figure 1. No repetitive response was observed at any stimulus interval less than 3.5σ and at 7.5σ interval the conditioned response still was only one-half of the unconditioned one.

In two other similar experiments the relatively refractory period was found to last for 15 and 17σ . Therefore, there can be no doubt that the recovery of nerve fibres is much slower than previously assumed.²

In the experiment illustrated in figure 1, 4, one hour later, when the nerve had been stimulated several hundred times the threshold of stimulation became 11 per cent higher and the relatively refractory period dropped first to 9 and somewhat later to 7.5σ . The absolutely refractory period measured with two supermaximal shocks remained constant at the ordinary figure of 0.56σ .

The refractoriness of the motoneurone-nerve-muscle preparation created by a maximal shock delivered to the motor nerve (antidromic shock). It has been demonstrated in a previous paper (Lorente de N6, 1935b) that the motoneurone when excited across synapses, if any, has an absolutely refractory period which can not be longer than that of the axon. Therefore it could be predicted that an antidromic shock would produce a refractoriness of the motoneurone-nerve-muscle preparation no longer than the refractoriness found in the nerve-muscle preparation. The experimental results have fully confirmed this prediction.

Figure 2 contains several curves constructed by plotting the height of the response elicited across the motoneurons against the interval in sigmas between the antidromic and the testing floor shocks. The potential developed by the maximal antidromic shock is indicated on the axis of the ordinates.

In obtaining curve 1 a maximal stimulus at the floor of the fourth ventricle was used. The response, which of course was being obtained across

² The extraordinarily long relatively refractory period (15 to 20σ) mentioned in text, can not correspond to the classical one; it must rather include the period of late subnormality described by H. T. Graham (1933, 1935). Gasser (1935) finds that subnormality develops in normal nerves when repetitive stimulation has created a positive after potential. In my own experiments the stimulation of the nerve was repeated once every one and a half second, so that the conditions for summation of positive after potentials were not present. Therefore it has to be concluded that Graham's period of subnormality may be developed in normal nerves after a single stimulus. However, I think it advisable to use the old denomination "relatively refractory period" for the whole interval of time during which the second response shows a deficit, until a new terminology is agreed upon.

the motoneurons, developed about $\frac{2}{3}$ of the potential of the maximal motor twitch. The earliest second response after the antidromic shock was obtained at 1σ interval; the recovery progressed very fast up to about 2σ interval and then slower; it became complete at about 13 or 15σ .

In obtaining curve 2 the stimulus at the floor of the fourth ventricle was reduced, so that the unconditioned response dropped to about $\frac{1}{3}$ of the

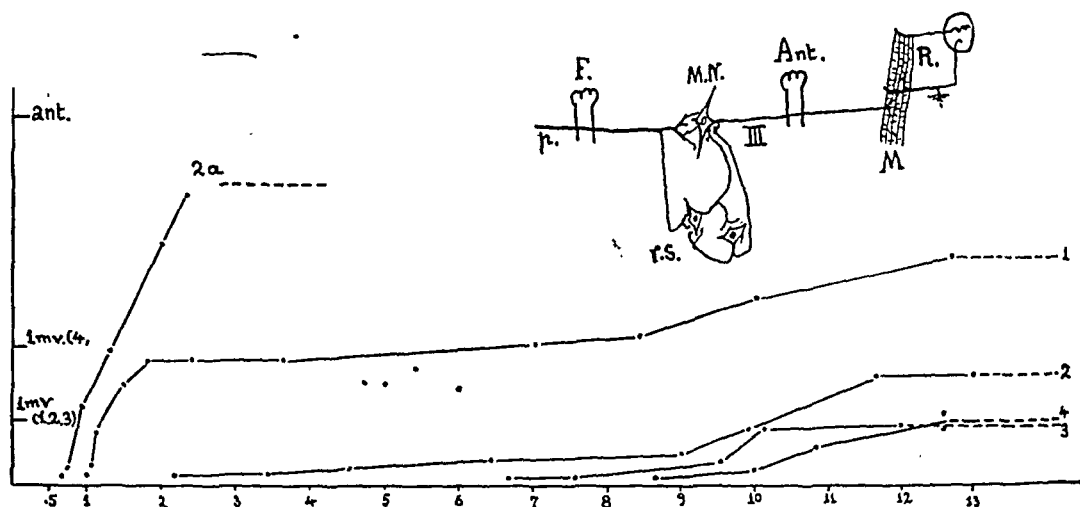


Fig. 2. Stimulating electrodes *Ant.* and *F.* on the oculomotor nerve (III) and on the floor of the fourth ventricle, on paths *p*, which end of the motoneurone *MN* and on reticular cells *r.s.* The responses illustrated by curves 1, 2, 3 and 4 were primarily due to the impulses set up in paths *p*, but the responses illustrated by curve 2a were facilitated by a previous floor shock which although subliminal for the motoneurons excited *r.s.* cells. Recording electrodes *R.* on the internal rectus muscle *M.*

The interval between the antidromic and the floor shock is given in abscissae in σ . The height of the response to the floor shock is given in ordinates (scale, 1 millivolt, at left). The potential developed by the antidromic response (maximal motor twitch) is given in the axis of the ordinates (*ant.*). The horizontal broken lines show the potential developed by the responses to the floor shocks alone. The antidromic shock was produced by discharge of $0.13\mu f$ condenser; potentiometer setting 0.15. The floor shocks were produced by discharge of a $0.14\mu f$ condenser, potentiometer settings 0,3; 0,17; 0,06; 0,05. In obtaining curve 2a two floor shocks were used at 0.6σ interval, the first produced by discharge of a $0.06\mu f$ condenser and the second by discharge of $0.14\mu f$ one; potentiometer setting 0.17. (expt. 9/1/35).

maximal motor twitch. Accordingly, the earliest second response was observed at about 2.2σ interval; the recovery was at first very slow; it increased rapidly after about 9σ and was complete at about 12σ .

In obtaining curve 3 the floor shock was still further reduced so that it set up a response of only about $\frac{1}{7}$ of the motor twitch. The earliest second response was not observed until the interval between shocks was as long as 6.7σ . The recovery was complete again at 13 to 14σ .

Finally in obtaining curve 4 a floor shock just above threshold was used, which elicited a response no more than $\frac{1}{10}$ to $\frac{1}{11}$ of the maximal motor twitch. The earliest second response was observed at 8.7σ interval; the recovery was complete at about 13 to 15σ .

Since even in the case of curve 1 the stimulus delivered to the motoneurones was relatively weak it was tried to increase it by facilitation, and the same floor shock as in curve 2 was used again, but preceded at 0.6σ interval by another facilitating subthreshold floor shock. The response thus obtained was considerably larger, almost 0.8 of the maximal motor twitch.

The earliest response after the antidromic one was obtained at an interval of 0.56 to 0.6σ between the antidromic and the second floor shock, and the recovery was complete at an interval of no more than 2.25σ (curve 2a).

Immediately afterwards the response to two supermaximal antidromic (i.e., motor) shocks was recorded and the curve of recovery of the potential of the second response was found to be practically identical with curve 2a in figure 2, which also is practically identical with curve 2 in figure 1.

On the other hand the similarity between curve 1 in figure 2 and 3 in figure 1 and between 2, 3 and 4 in figure 2 and 4 in figure 1 is very patent. It indicates that the process of recovery in both cases is very much the same and therefore the preparation motoneurone-nerve-muscle has the same refractoriness as the ordinary nerve-muscle preparation.

The fact that the curves 2a, 1, 2, 3 and 4 in figure 2 start the later the weaker is the stimulating shock, in other words, that the "absolutely" refractory period of the preparation depends on the strength of the stimulus at the floor of the fourth ventricle seems to have only one explanation. It has been shown in previous paragraphs that the fibres of the oculomotor nerve after conducting an impulse become partially refractory during 15 to 20σ , the refractoriness decreasing steadily after its start. The fact now that the motoneurones when weakly stimulated across synapses begin to overcome that refractoriness later than when strongly stimulated indicates that the change induced in them by the stimuli arriving to their synapses may have different strengths. In other words, the intensity of the excitatory process in the neurone can be measured by its ability to set up an impulse in the partially refractory axon. Since by varying the strength of the shock delivered at the floor of the fourth ventricle only the number of stimulated fibres ending upon the motoneurones is varied, it may be concluded that the degree of excitation of the motoneurones depends on the number of activated synapses.

The excitatory process of the motoneurones may attain all degrees, from maximal, when it is able to set up an impulse in an axon immediately after the absolutely refractory period, to threshold-value, when it does not start an impulse until the very end of the partially refractory period.

The dependence of the strength of the excitatory process on the number of activated synapses explains a noteworthy difference between curves 3 and 4 in figure 1 and curves 1, 2, 3 and 4 in figure 2. The curves obtained from the ordinary nerve muscle preparation never have sudden steps and as a rule they end the later, the weaker the testing shock. But when the excitation takes place across the motoneurons the curves have definite steps with almost horizontal stretches between each two of them. On the other hand it is often found that in spite of wide variations in the intensity of the stimulus, they may become horizontal nearly at the same interval between shocks (curves 2, 3 and 4, fig. 2).

This is easy to understand. The curves obtained with the nerve muscle preparation, when the testing shock is weak really represent the statistical distribution of electrical thresholds (or recoveries) in the nerve. In the case of the motoneurone-nerve-muscle preparation the shape of the curve is determined by two factors: the statistical distribution of thresholds in the motor axons and the statistical distribution of synapses on the motoneurons. The latter is dependent on the branching of fibres within the

Fig. 3. Stimulating and recording electrodes as in figure 2. The records show the lack of effect of an antidromic impulse on facilitation. Records 5 to 11 have been used in constructing table 1, and records 14-20 in constructing curves 1, 2 and 3 in figure 4.

1. Timing film ($1/2\sigma$) for records 2 to 11. 2. Response to a maximal antidromic (motor) shock. 3. Response to two maximal antidromic shocks at 0.72σ interval; the second shock elicits one of the earliest second responses (absolutely refractory period in that preparation 0.60σ). The amplification in records 2 and 3 is slightly less than in records 4 to 11. 4. Two floor shocks at about 1.3σ interval, the second one giving rise to a greatly facilitated response. Note the difference (0.8σ) in latency in records 2 and 4, which, less conduction time from electrodes *F* to electrodes Ant., measures the synaptic delay in the motoneurons. 5. The antidromic shock followed by the second floor shock, which remains ineffective. 6. Same shocks as in 5 but preceded by the facilitating first floor shock. The response to this shock is obliterated by the antidromic impulses, but the second floor shock elicits a response. Distance between antidromic and second floor shock 0.43σ . In records 7, 8 and 9 this distance is increased and an increase of the second response results. 10. Two floor shocks at 0.65σ interval. 11. The same floor shocks, but the first one preceded 0.1σ by a maximal antidromic, which obliterates the first floor response but does not prevent a response of some facilitated motoneurons to the second floor shock. 12. Timing film (1σ) for records 14 to 17. 13. Timing film (1σ) for records 18-20. 14. Second floor shock alone. 15. Both floor shocks; the second response is facilitated. 16. A maximal antidromic shock placed between the two floor ones; the response to the first floor shock is obliterated, but the response to the second one is only slightly decreased. 17. The second floor shock preceded only by the antidromic. No response is obtained. 18. Two floor shocks, the second one giving a greatly facilitated response. 19. A maximal antidromic shock followed by the second floor shock. No response to the latter is observed. 20. The antidromic shock is preceded by a facilitating floor shock. The second floor shock produces now a strong response.

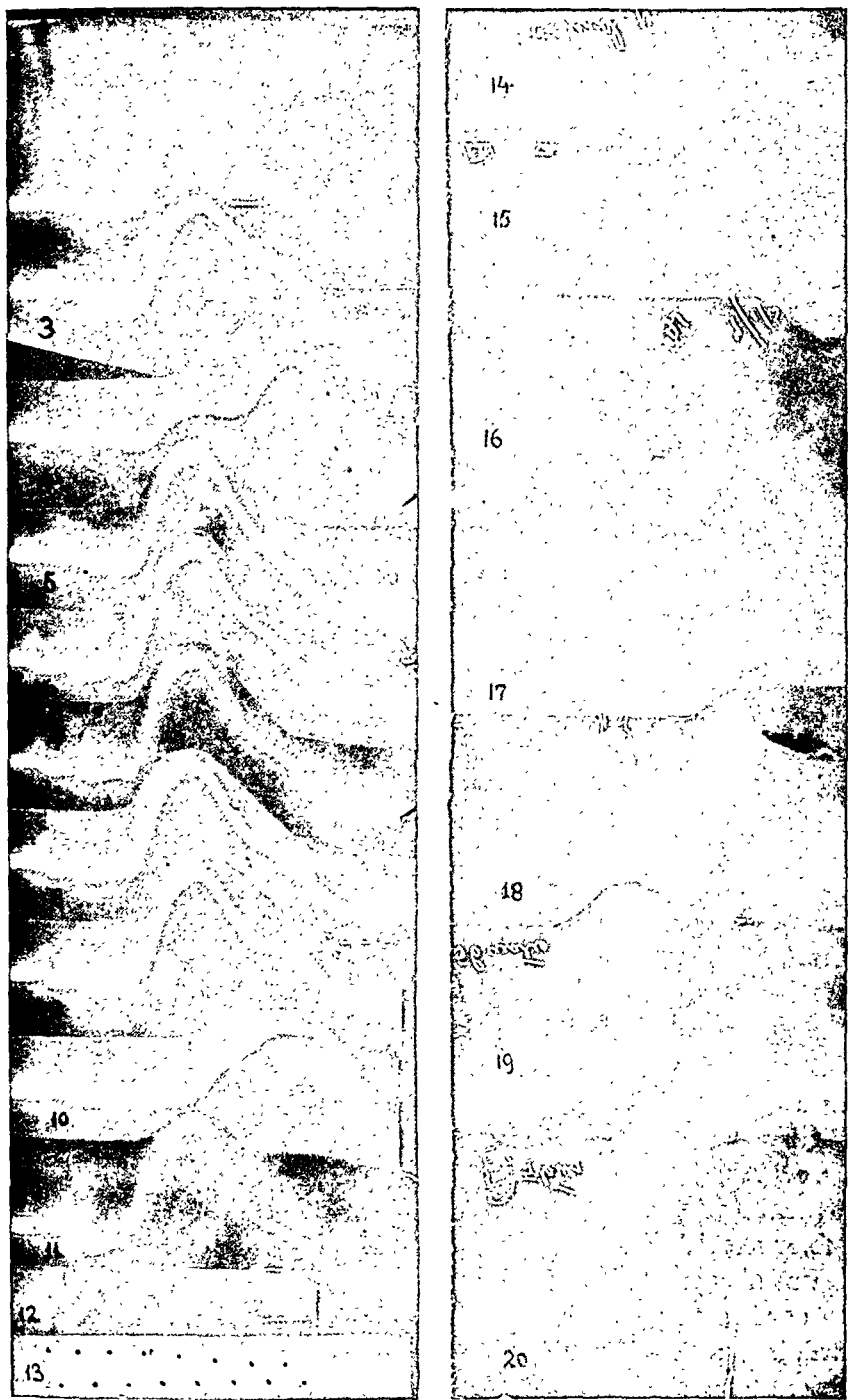


Fig. 3

motor nucleus, and therefore it may happen that following a certain shock at the floor of the fourth ventricle neurones with axons of high electrical threshold have many activated synapses while neurones with axons of low electrical threshold have only a few. For the floor shock both kinds of motor axons will have the same threshold.

The effect of an antidromic shock on facilitation. When a shock delivered through electrodes placed on the floor of the fourth ventricle is preceded at a suitable interval by another shock through the same electrodes a facilitation of the response to the second shock is produced, which may be demonstrated as well when both shocks are subliminal as when both are liminal. The temporal course of facilitation varied from preparation to preparation but remained constant in its general traits. In many cases it lasted sufficiently to make it possible to intercalate an antidromic shock between the two floor shocks.

The results of a considerable number of experiments have been absolutely constant. In no case has it been possible to demonstrate that the antidromic impulse had destroyed the facilitating effect of the first floor shock. The modification of the facilitated response was apparently due to the refractoriness created in the motor fibres by the antidromic shock.

Figure 3 reproduces several records and figure 4 contains several typical curves illustrating the recovery of the response to stimuli at the floor of the fourth ventricle after an antidromic shock. Curves 1, 2 and 3 in figure 4 belong to the same experiment. Two maximal floor shocks were used, each one alone giving rise to a response which developed the potential P_2 . When facilitated the second shock gave rise to a response with potential P_1 , almost $\frac{9}{10}$ of the potential P of the maximal motor twitch.

Curve 3 illustrates the recovery of the response to the second floor shock when preceded only by the antidromic one. No second response was observed until the interval between shocks was 1.45σ . The second responses increased in size very slowly and were not entirely recovered at an interval between shocks of 7σ . Measured in terms of the refractoriness it was able to overcome, the excitatory process of the motoneurones created by the unconditioned floor shock was rather weak.

Curve 2 illustrates the response to the second floor shock when preceded by the facilitating floor one and the antidromic. The distance between both floor shocks (marked by arrow 2) was 4.4σ , so that the antidromic shock was always placed between them.

The earliest response to the second floor shock was obtained at 0.6σ interval, i.e., almost immediately after the nerve fibres had recovered from the absolute refractoriness created by the antidromic impulse. Therefore the antidromic shock had not destroyed the facilitation created by the first floor shock, which raised the excitatory process created by the second floor shock to maximal value. The recovery of the facilitated response

proceeded then very fast and became complete at about 5σ . Curve 2 is at every shock interval considerably higher than curve 3.

In obtaining curve 1 the distance between both floor shocks was reduced to 2.45σ . The results were again the same. The antidromic shock never destroyed the facilitating effect of the first floor shock.

In another typical experiment, the distance between floor shocks was reduced to 0.8σ . The antidromic shock at the beginning was placed between and later preceding both floor shocks. Facilitation was present at every interval between shocks and the curve plotted as above did not show any break when the antidromic shock was simultaneous with the

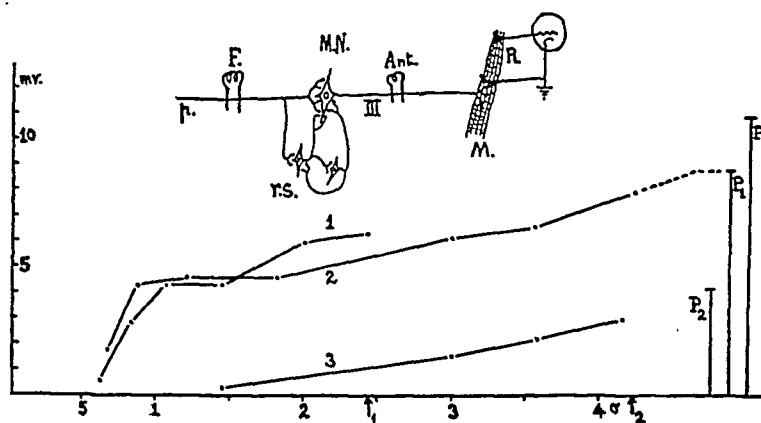


Fig. 4. Stimulating and recording electrodes as in figure 2. The experiment was arranged as indicated by records 14-20 in figure 3. The curves are a plot of the height of the response to the second floor shock when preceded only by the maximal antidromic (3) or by facilitating floor and a maximal antidromic (1, 2) (expt. 7/1/35). The interval between the antidromic and the second floor shock is given in abscissae. Arrows 1 and 2 indicate the constant interval between the two floor shocks in curves 1 and 2 respectively, so that the interval between the first floor shock and the antidromic has to be read from the arrow toward the origin.

P , potential of the antidromic response; P_2 , potential of the response to the second floor shock alone; P_1 , potential of the response to the facilitated second floor shock.

facilitating floor one. Undoubtedly, even in that case the facilitation remained unaffected by the antidromic impulse.

The same results have been obtained in all the other experiments (five) in which the facilitation was strong enough to raise the excitatory process to maximal value in a number of motoneurons. In one experiment in which a large number of motoneurons were excited maximally by the facilitated second floor shock, it was tried to determine the shortest possible interval between antidromic and second floor shocks, at which a repetitive response appeared. Several of the values are given in table 1. Figure 3 contains a few of the records obtained.

It is evident from the previous experiments and from this table that the

position of the antidromic shock in relation to the facilitating floor one is of no consequence; the appearance of a second response depends solely on two factors: *a*, the distance between floor shocks, which determines the amount of facilitation and therefore the strength of the excitatory process of motoneurons, and *b*, the distance between the antidromic shock and the testing floor one, which determines the degree of recovery of the axons after the antidromic impulse.

TABLE 1

Intervals in σ between a conditioning floor shock (F1), a maximal antidromic shock (Ant.) and a testing floor shock (F2) at which a second response due to F2 was observed

If F1 was omitted, F2 remained ineffective (expt. 11/1/35). Values $\pm 0.02 \sigma$.

F1	0.62 σ Ant.	0.62 σ F2
F1	0.51 Ant.	0.85 F2
F1	0.80 Ant.	0.43 F2
F1	0.70 Ant.	0.60 F2
F1	0.13 Ant.	0.68 F2
F1	0.00 Ant.	0.65 F2
Ant.	0.10 F1	0.65 F2

DISCUSSION. The main problem to be considered now is whether or not the antidromic impulse enters into and modifies the state of the body and dendrites of the motoneurons. Sherrington (1900, p. 798) and Eccles and Sherrington (1931) take it for granted that the antidromic impulse passes into the neurone body and dendrites and is first detained at the synapses. But recently Forbes (1934) argues that the antidromic impulse may be stopped at the axon hillock and therefore may not affect the motoneurone at all.

It is true that the antidromic impulse does not seem to start a new potential in the cell body (Gasser and Graham, 1932; Hughes and Gasser, 1934), but of course a qualitatively new potential could hardly be expected. On the other hand it is difficult to visualize how an antidromic impulse could be stopped at the axon hillock, where the axonal and the cell membranes are continuous; it is the more difficult to understand as there are cases in which it is positively known that the impulses pass through the body of the nerve cells. For instance, the ganglion cells of Scarpa and Corti are bipolar and therefore the impulses originated in the internal ear have to pass through their bodies. Certainly there are some histological differences between the ganglion cells of the eighth nerve and the motoneurons but whether those differences might account for such a radical change in functional activity is so doubtful, that only an extremely well

controlled evidence would justify the assumption that the antidromic impulse does not penetrate into the motoneurone. After all the body and the dendrites of the neurone have to be considered only as a specialized part of the axon, adapted to the function of receiving the excitatory impulses. That the body contains the nucleus and certain other structures does not oppose this assumption because often enough the body of the nerve cell lies clearly outside the path of the impulses (Cajal, 1909, 1911, 1934).

On the basis of this assumption it is easy to understand that an antidromic impulse does not produce any other result than to make the cell refractory and does not affect facilitation because the facilitating and in general the excitatory mechanisms are located outside the motoneurone.

In the presentation of the experimental results no reference has been made to the synaptic delay. The existence of a synaptic delay signifies that impulses arrived at the synapsis set up a new impulse in the axon of the motoneurone after a certain period of time, and it is very noteworthy that the synaptic delay is not lengthened after arrival of an antidromic impulse. At first the refractoriness created by the latter prevents the setting up of a new impulse, but when the earliest response is obtained, it is set up after the customary synaptic delay.

The comparison of records 2 and 4 or 10 in figure 3 shows that the synaptic delay in that preparation measured about 0.60 to 0.65σ , because the distance between the *F* and the *Ant.* electrodes (fig. 2) was about 20 mm., and the comparison of record 4 with records 6, 7 and 8 demonstrates that the synaptic delay was practically unchanged even in the earliest second response of the motoneurones, because the second crest in records 6, 7 and 8 is found very nearly in the same position as in record 4. As a matter of fact, it seems to have moved forwards. Since as record 3 shows, the early second responses in the nerve-muscle preparation are considerably delayed one should be inclined to think that after an antidromic impulse the synaptic delay of the motoneurones is shortened; at any rate it is not lengthened.

The shortest possible interval between an antidromic and an effective floor shock has been determined to be 0.43σ ; adding to it the synaptic delay (0.60 to 0.65σ) it results that the floor shock has set the earliest second impulse in the axon about 1.15σ after the antidromic one, i.e., about 0.5σ later than a maximal induction shock delivered to the nerve (see fig. 1, 1 and 2). This explains why the earliest second crest in the motoneurone-nerve-muscle preparation (records 5, 6, 7) appears somewhat later than in the ordinary nerve muscle preparation. However it has to be mentioned that in other experiments in which on the one hand the stimuli created by the floor shocks were stronger (because they produced, without facilitation, maximal motor twitches), and on the other hand both impulses had to pass

through the motoneurones the second responses appeared as early as in the ordinary nerve muscle preparation (compare fig. 3, records 2 to 11, with figure 2 in Lorente de N6, 1935b).³

Under such conditions it is evident that the mentioned shortest possible interval of 0.43σ measures the upper limit of the absolutely refractory period of the dendrites and cell body for the particular strength of stimuli used in that experiment. As soon as the cell body (i.e., thickened or receptive end of the axon) recovered from absolute refractoriness the impulses which arrived at the synapses were able to set up an impulse in the axon, after the ordinary synaptic delay. And that figure 0.43σ also is the upper limit of the time during which the excitatory process at the synapsis remains at full strength, because if it should remain at full strength for a longer period of time the interval between the antidromic and the earliest effective floor shock would be shorter.

With the determination of that low value (0.43σ) for the shortest interval between antidromic and earliest effective floor shock a direct proof is given of unidirectional conduction across the central synapse. The distance between the two stimulating electrodes (*Ant.* and *F*, fig. 2) was about 20 mm. Even assuming that the antidromic impulse had been conducted through the third nerve and after crossing the synapse through paths *p* at a speed of 100 m. per second it could not have reached electrodes *F* earlier than 0.2σ after the antidromic shock. Under such conditions it would remain only 0.23 for the absolutely refractory period of the *p* fibres. It is evident then that the antidromic impulse does not cross through the synapses. With these experimental data the question as to the ultimate cause of the dynamic polarization of the neurone (Cajal, 1891, 1900; v. Gehuchten, 1900) is decided in favor of Sherrington's assumption (1900) of a block of the impulse at the synapses.

The concept of the neurone as a nerve fibre provided with a trophic centre and two specialized endings affords satisfactory means of understanding the rôle of the intercellular connections within the nerve centres, as discussed in other papers (Lorente de N6, 1934) because the thickened part of the axon (body and dendrites) also will have the two types of responses known to exist in the ordinary nerve fibres (Lucas, 1917): local responses capable of summation and conducted or all-or-none responses.

In closing these theoretical remarks it has to be mentioned that in early days the existence of "local" summation at certain points of the neurone

³ The comparison made in a previous paper (Lorente de N6, 1935b, page 287) of the earliest second responses in the nerve-muscle and in the motoneurone-nerve muscle preparation is not entirely correct because with the former (fig. 1 in that paper) only submaximal shocks were used, which of course did not excite the motor fibres immediately after the absolutely refractory period, but some time afterwards. The comparison made in later experiments under identical conditions for both preparations has shown that there is no measurable delay of the second impulse during its passage through the dendrites and body of the neurones.

was postulated by Sherrington (1925, p. 524) and that the interpretation given by Hughes and Gasser (1934) of the potentials found in the spinal cord during activity, clearly is based upon the same concept used above, that the neurone body has the same elemental properties as the axon.

SUMMARY

The effect of an antidromic impulse on the response of the motoneurons has been studied in a preparation in which the motoneurons receive stronger stimuli than through the ordinary reflex arc.

The antidromic shock does not produce any other effect than to make the axon refractory; it does not destroy facilitation.

The refractoriness of the axon, also measured in the ordinary nerve muscle preparation lasts for about 20σ . This long refractoriness is interpreted as being due to the sub-normal period of nerve discovered by H. Graham (1933, 1935) and again found by Gasser (1935).

The ability of the neurone to overcome the depression of the excitability of the axon gives a measure of the strength of the excitatory process created at the synapses.

A direct proof of unidirectional conduction across the central synapse is given.

The neurone is interpreted as a nerve fibre having two specialized endings, the receptor and the effector ones. The trophic centre with the nucleus may be located within the fibre itself and acquire the shape of cell body with dendrites.

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DETERMINATIONS OF REFRACTORY PERIODS IN THE TURTLE HEART

A. S. GILSON, JR.

*From the Department of Physiology, Washington University School of Medicine,
Saint Louis*

Received for publication May 7, 1935

There is an extensive literature dealing with the responsiveness of cardiac muscle to electrical stimuli and the changes of excitability manifested during the cardiac cycle, under the influence of vagus or sympathetic stimulation, of drugs, and so forth. A critical review of this literature would demand consideration not only of such variables as these mentioned but also of the various experimental conditions involved, such as changes in heart rate and in form of the stimulating shock used. A recent study of changes in electrical excitability changes during the cardiac cycle has been made by Eccles and Hoff (1934). Although they attempted to restrict themselves to the pacemaker of the cat's heart, the curves which they present resemble, in general, those which might have been plotted for other parts of the heart. In considering any series of excitability curves of this sort one should continually bear in mind the fact that changes of excitability may result from a variety of causes and that the changes which appear are in some cases capable of algebraic summation. Therefore conclusions as to the effects of any single procedure, such as vagal stimulation, are valid only if all other conditions, notably heart rate, be maintained constant. Fundamental though this requirement may be it has at times been overlooked. Furthermore, differences in form and duration of the shocks used may result in differences in the results obtained and consequently in the conclusions drawn from the experiments performed.

There have recently been described (Gilson, 1935) certain striking differences in the apparent cyclic changes in excitability in normal and in vagus-inhibited turtle hearts according as to whether the excitability is measured by induction shocks of short duration or by double condenser charges of much longer duration. The difference between the results obtained by the two methods seemed sufficiently striking to warrant reinvestigation of the changes in excitability during the cardiac cycle and also of the changes in form of the excitability curves with changes of rate and with vagus inhibition. Certain results obtained in this investigation are presented in this paper.

Determinations of refractory phases in the turtle ventricle. Determinations of excitability as measured by induction shocks were attempted some years ago using the quiescent, excised turtle ventricle. Stimulation was accomplished by breaking a spring switch which was demonstrably variable in action. The results obtained indicated that following a response of the ventricle there was a period of increasing excitability which lasted over a period of many seconds. Determinations were then attempted by means of a device similar in basic concept to that used by Andrus and Carter (1930). A lead was made from the electrocardiograph amplifier to a calibrated delay mechanism. This involved the discharge of a negative bias on the grid condenser of a gas-filled triode. The resulting activation of this triode caused the closing of a relay switch which in turn allowed the discharge of a condenser through a Thyatron. This Thyatron shock was used as a stimulus directly in a few experiments but in most instances was passed through the primary of a Porter coil and the shock from the secondary coil was used as the stimulus. In either case, control of the shock strength was accomplished by a voltage divider the derived circuit from which included the stimulating electrodes and the tissue. By making connections from the exposed, intact and beating heart to the amplifier, the R- or S- wave of the electrogram could be made to commence the series of events which terminated with the delivery to the heart of a shock of desired strength applied at any desired time. The results obtained with this technic were in general similar to those obtained by Eccles and Hoff. Threshold values or times were not well reproduced, especially after the use of shocks of four or five times threshold for determination of the earlier points on the recovery curve. The forms of the plotted curves varied considerably in different preparations. No definite "super-normal" phases were plotted from our data, but several preparations yielded curves which were quite flat during the latter part of diastole.

Recently in experiments using double condenser charges (fig. 1) as the stimulus, rather different and much more consistent results have been obtained. The stimuli used in most experiments had a time to maximum voltage of 0.018 second. This time constant may be changed through a rather wide range (0.003 sec. to 0.150 sec.) without changing the relative values along the curves plotted if determinations for any given curve are all made with a stimulus of fixed form. However, the absolute voltage values of the threshold stimuli plotted may be significantly altered by change in form of the stimulus. In these experiments, the use of the action potential as a means of activating the stimulating mechanism was discarded since it multiplied the technical difficulties of making determinations and since the use of a manually operated switch yielded results having a degree of accuracy which was sufficient for the present work. As the stimulator was arranged during the course of the experiments reported

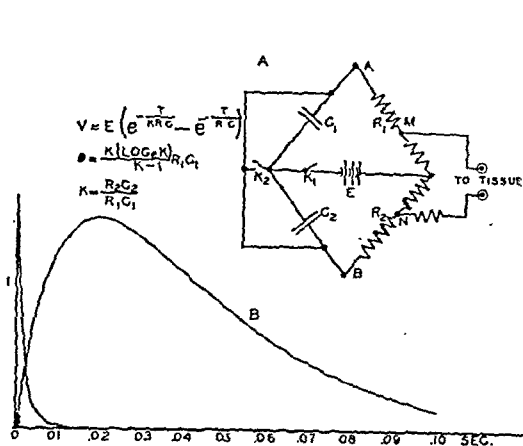


Fig. 1

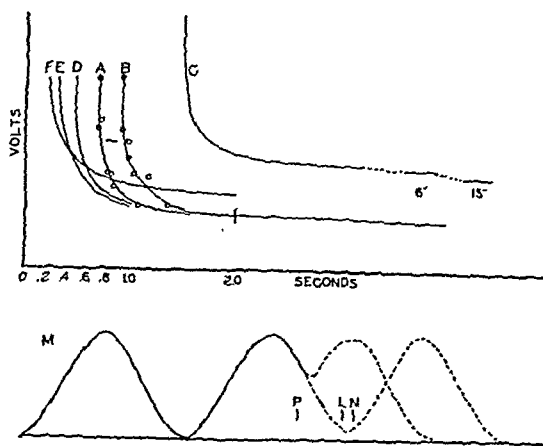


Fig. 2

Fig. 1. A. Diagram of circuit used for stimulation by double condenser charges.

B. Curves showing the form of such a stimulus as compared with the form of a shorter single condenser shock as obtained directly from our Thyatron stimulator and with the approximate duration (not form) of an induction shock.

For the double condenser charge plotted here, R_1 and R_2 were each 55,000 ohms, C_1 was of 0.25 and C_2 of 0.5 mfd. capacity; $\theta = 0.018$ second. The maximum voltage between M and N was 0.0256 volt for every volt applied by the battery, E. For refractory period determinations, the switches were complicated to allow a second shock to be applied through the same network.

Fig. 2. Curve M. Diagram of the mechanical response of turtle ventricle indicating the conditions under which curves A and B were obtained. Following two normally activated beats of the ventricle, if S1 were not applied, a normal systole would have commenced at time N. Application of S1 approximately at the time P elicits an early premature systole. A plot of threshold values of S2 at various times following S1 so placed yields the curve A. Measurements of excitability following a systole elicited by S1 placed at time L yield data from which B was plotted.

Curve A. Curve of recovery of excitability following an early premature systole of ventricle of normally beating turtle heart.

Curve B. Same but for late premature systole. The curve is extended on a basis of data obtained on the non-rhythmic ventricle and on the ventricle of the heart in standstill under the effect of vagus inhibition (see text).

Curve C. Curve of recovery of excitability plotted for excised, quiescent ventricle. Same preparation as for A and B but $\frac{1}{2}$ hour to 1 hour later. No perfusion.

Curves D and E. Excitability curves plotted for atrium after late and early premature beats, respectively.

Curve F. Excitability curve plotted for late premature beat of atrium under vagal inhibition. The left vagus was stimulated. There was no change in rate of the heart.

To avoid confusing the curves, points actually determined are given only for curves A and B. About the same number of points having about the same deviation from the smoothed curve was obtained in each case.

Curves A, B and C are plotted directly from recorded data. For purposes of comparison, curves D, E and F have been plotted as if the late diastolic threshold of the uninhibited atrium had been the same as that of the normal ventricle. Actually the threshold determined for the atrium in this experiment was somewhat above that for the ventricle.

The raised threshold for curve C is not to be attributed merely to the fact of excision of the ventricle. An excised ventricle may show either an increase or decrease of threshold values depending upon the resultant of a number of factors of which some are physiological and others are physical.

here, the operation of a double-throw, double-pole switch delivered one stimulus (S1) to the heart and commenced the chain of events which resulted in a second stimulus (S2), of desired strength, being delivered at a predetermined time thereafter. S1 caused the systole, following which the course of the recovery of excitability was determined. Since varying the time in the ventricular cycle at which S1 was delivered, significantly changed the course of the plotted curve obtained from the time and S2 data, two recovery curves are given, for example curves A and B of figure 2. Of these curves, A represents the course of recovery after the beat obtained when S1 is thrown in early in diastole. The other curve, B, is obtained when S1 is thrown in late in diastole, thus giving a nearly normal systole for the heart beating at its normal rate. To stimulate early in diastole, it is necessary that S1 be considerably above a minimal stimulating value but the use of this stronger stimulus has been demonstrated not to change the form of a "late" curve from that which would have been obtained with a minimal strength for S1. Another possible source of error was that a time error in placing S1 will make a difference in the position of the corresponding point on the recovery curve determined by the S2 shock. However, it is probable that S1 can be placed with an error of less than 0.1 second and it is a fact that the position of a given strength of S2 just above threshold is reproducible to within 0.03 second and usually to within 0.01 second error when checks are made immediately. Over a longer period, fluctuations of thresholds may change absolute values somewhat though relative values remain without significant change for periods of one to two hours. For a heart beating with an intersystolic interval of about 1.5 seconds, as is the case in most of our experiments, the errors introduced by the methods used are therefore less than the error which could be tolerated at the present stage of our knowledge.

Figure 2 presents curves (A, B and C) plotted from the data of a typical experiment. The brain of the turtle (*Pseudemys elegans*) was pithed, the plastron and front legs were removed, the vagi cut high in the neck, and the pericardial sac was opened widely. The stimulating electrode was a silver, ball-tipped wire, the contact face being flattened and having a diameter of about one millimeter. This was placed lightly against the surface of the heart. For this experiment, the electrode was placed against the ventral surface of the ventricle about one-third of the way from the apex to the basal margin. An indifferent silver electrode was placed in the intestinal mass. Curves A and B were obtained with the intact, spontaneously beating heart. The temperature was 26.5°C., the intersystolic interval 1.6 second. The strength of S1 (60 volts) is indicated by the short horizontal line. For every volt applied to the bridge (the values recorded) something less than 0.026 volt maximum was applied to the electrodes. The curves show in each case the earliest time at which an S2 stimulus of

voltage as plotted along the ordinates will elicit a response. Curve *A* is plotted from points made with S1 early, curve *B* with S1 late in diastole. Curve *A* is seen to lie below curve *B* for all points plotted. With the beating heart in good condition this is the result which we have always obtained. The amount of separation of the curves given here is somewhat greater than average.

Curve *C* was obtained from use of the excised, non-rhythmic ventricle and determinations were begun about half an hour after completion of determinations for *A* and *B*. It is seen that in comparison with the earlier determinations made on the ventricle of the intact heart, these later determinations show that the thresholds were increased and that the absolutely refractory period was increased. The general form of curve *C* is similar to that of curves *A* and *B*. For threshold determinations with the shorter S1-S2 intervals (up to 2.6 sec.), stimulation was maintained at regular intervals with 10 second pauses between successive S1 stimuli. The use of the non-rhythmic preparation makes possible the demonstration of two facts not to be seen in the beating heart. The first of these is the continued increase in excitability which can be followed almost invariably for 20 seconds and frequently for as much as 30 seconds. In this experiment only two such late points were particularly located, one for a stimulus of 45 volts which was effective after 15 seconds and not after 10 seconds, the other for a stimulus of 48 volts effective after 6 seconds but not after 5 seconds. The positions of these points were not due merely to casual fluctuations in excitability but were in each case checked by repetition of determinations. With respect to the second fact, it has been found that in curves *A* and *B* the curve of recovery from an early premature systole (curve *A*) lies below the recovery curve determined for a late systole (curve *B*). However, determinations made on the non-rhythmic ventricle show that at later times the recovery curves usually cross so that the earlier relationship is reversed. As specifically determined in this experiment, at a time 2.6 seconds after an S1 stimulus, a 52 volt stimulus was effective when the ventricle had had a previous resting interval of 30 seconds but was ineffective when the resting interval had been but 10 seconds. The precise course of the curves in this and the still later portion depends upon a number of factors, the delay in recovery becoming greater for example with a fatigued or anoxic preparation.

Stimulation of the vagus produced no change in ventricular excitability as a primary effect. If vagus inhibition stopped the heart, a systole was followed by a period of rising excitability which continued for many seconds in a manner similar to that seen with the non-rhythmic ventricle. This continued rise of excitability was such that the final threshold values were between 10 per cent and 20 per cent lower than the lowest values obtained on the normally beating ventricle. The apparent increased

excitability is therefore considered as being due merely to the standstill of the heart and not to any primary effect of the vagus upon the turtle ventricle.

Determination of refractory phases in the atrium. The refractory phase determinations made on the turtle atrium yielded curves similar to those plotted for the ventricle (fig. 2, *D* and *E*). The absolutely refractory phase of the atrium is significantly shorter than is that of the ventricle. Difficulties due to stimulation of vagus fibers in the atrium usually did not interfere with normal determinations if several beats were allowed between successive determinations.

Early in the course of this work, an attempt was made to determine changes of excitability in the atrium during periods of vagus inhibition by means of induction shocks. The shortened period of absolute refractoriness reported by Lewis, Drury and Bulger (1921) and by Andrus and Carter (1930) for the partially inhibited atrium was easily demonstrated. However, no consistent change in the late diastolic excitability could be demonstrated in the inhibited atrium which was beating at a rate unchanged from the normal. Referring now only to determinations made with induction shocks applied late in diastole, it may be said that in some preparations vagus stimulation produced no measurable change in excitability, in other cases there was a rise of threshold, in still other cases there was a fall of threshold. In some preparations only the increase was found, in other preparations only the decrease was found and in still others determinations at different times gave at one time an increase and at another time a decrease of threshold. In no case did we measure a change of threshold of over 5 per cent and in only occasional instances was the measured change over 2 per cent.

Since the use of such short induction shocks yielded only such negative or negligible results, we sought for other means of stimulation which might yield results more consistently correlated with the obvious functional depression appearing in the heart during periods of vagus inhibition. Two groups of facts seemed to have significance in this connection. First, there was the statement repeatedly made in the literature and best demonstrated, so far as we are aware, by the data of Ashman and Garrey (1931) that there is a rise of the rheobasic current necessary for stimulation of the vagus-inhibited as compared with the normal atrium. Secondly, there are the relationships presented by Monnier (1934) in his exposition of the concept of paramediation. Monnier's development of this concept is a logical outgrowth of the discovery by Lapicque (1908) that two double condenser charges of different and suitable forms, respectively, may be used to stimulate selectively the nerve from a toad or the nerve from a frog. Monnier has greatly extended the theoretical and experimental investigation of stimulation by double condenser stimuli. He reports that

for a double condenser stimulus of optimal form for stimulation of a frog nerve (1) the time to maximal voltage of the stimulus is equal to the utilization time for the nerve, (2) that the threshold voltage for a stimulus of this form is only slightly more than the rheobasic voltage for a rectangular constant current, and (3) that the nerve stimulated by such a wave at just threshold voltage produces a response of the nerve which begins at or very close to the crest of the wave. It seemed obviously desirable, therefore, to attempt the use of such stimuli. In preliminary determinations the stimulator used had the values $K = 2$ and $\theta = 0.018$ second (see fig. 1). The value $\theta = 0.018$ second was obtained by employing such resistances and condensers as were immediately available in the construction of a stimulator having a θ value approaching the utilization time of 0.02 to 0.025 second previously found by Gilson and Peugnet (1932) for turtle ventricular muscle.

The use of such stimuli to determine excitability in the heart during periods of vagus stimulation which caused no change in rate, revealed a marked and invariable rise above normal of the late diastolic threshold values *if these were determined on parts of the heart showing functional depression as a result of the vagus stimulation*. Whether the differences observed between the results as obtained with induction shocks in the one case and with double condenser charges in the other case are primarily due to shock form differences or to the high voltages necessary for stimulation with the short induction shocks, we are not at present able to say. However, the reproducibility of threshold determinations made with the double condenser stimuli has been so much greater than was formerly possible with induction shock determinations that in recent experiments we have confined ourselves to the use of the former. The results of experiments in which only this type of stimulus was used are reported below in more detail.

If the left vagus is stimulated so as to cause a marked inotropic depression of the atria without change of heart rate, the curve of the recovery of excitability following a contraction is significantly changed from the normal. The absolutely refractory period is shortened and recovery from relative refractoriness is greatly slowed (fig. 2, curve *F*). If moderate vagus stimulation results in a slowed or stopped heart, as may occur with right vagus stimulation, the slowing of the rate results in a lengthening of the absolutely refractory period. Furthermore, a single threshold determination made after a long resting interval may approach or achieve a normal late diastolic value. Thus it is possible, under such conditions, to obtain single point determinations which show no change from normal values. With standstill resulting from more intense vagal stimulation, the delay of recovery is so great that determinations made as much as 30 seconds after a previous beat will still show elevation of threshold. For

atria beating with unchanged rate but showing moderate inotropic depression as a result of vagus stimulation, threshold determinations made late in systole have regularly shown increases of from 50 per cent to 75 per cent. Valid increases of 100 per cent to 200 per cent have probably been observed but in these cases the inotropic depression of the stimulated atrium has been so intense that no visible contraction could be observed, the only sign of response being that at another part of the heart. It is believed that these responses were due to effective stimulation at the electrode followed by conduction in the normal manner to other parts of the heart. However, the use of suitable recording means will be necessary before such findings can be considered as adequately demonstrated.

DISCUSSION. Attempts to determine changes in the functional state of excitability of the heart by means of thresholds to induction shocks have not been satisfactory. The results of such experiments lead one to choose between two possibilities. The first of these is the belief that electrical stimuli do not measure the functional state of excitability. This is the conclusion reached by Eccles and Hoff with regard to the pacemaker of the cat heart. The other possibility is that an induction shock is not an adequate or valid form of stimulus to use in measurements of cardiac excitability. For the present we are inclined to accept the latter alternative. Further experiments may show that this latter alternative is not exclusive of the former. However, we have been struck by the closeness of agreement between the apparent functional state of the heart and the thresholds to double condenser stimuli under the conditions with which we have worked. With respect to the agreement between our experiments and those of former workers, we may point out that there is general agreement of the results in those cases in which the experimental procedures were similar. That our conclusions differ from those generally accepted at the present time is due to the fact that the stimuli which we have employed give different results from those obtained with induction shocks.

If we define, for use in this paper, the word *excitability* as "a reciprocal function of the maximum voltage of a double condenser stimulus such as has been described above and of strength just sufficient to elicit a response at the moment considered," the following synopsis may be presented. For the ventricle of the turtle there is a period of complete inexcitability which probably coincides in time with the period of systole at any given point. Following this period of absolute refractoriness, there follows a period of relative refractoriness during which the excitability rises. In a normal, spontaneously beating heart, the ventricular excitability is still rising at the time of arrival of the next normal impulse. If the data obtained from the excised, quiescent ventricle and from the ventricle of a heart in vagus standstill be considered, the curves obtained with the beating ventricle might be extended. From such an extended curve we

would conclude that, following a systole, the excitability continues to rise for a period of 20 to 30 seconds and reaches a level which is probably 10 per cent to 20 per cent above that attained by the intact ventricle of the beating heart. The period of absolute refractoriness is shorter following an early premature beat than it is following a normal or slightly premature beat. This difference is comparable with the difference to be seen in a rapidly beating as compared with a slowly beating heart under otherwise identical conditions, the rapidly beating heart having a shorter and the slowly beating heart a longer period of systole. The very late part of the recovery curve may show a delayed recovery in the case of the early beat. This last factor does not appear in the curves of the ventricle of a normally beating heart in good condition. It may become significant in a sufficiently depressed heart and may usually be seen in the excised quiescent ventricle.

Concerning the atrium, a somewhat similar statement could be made. The absolutely refractory period is much shorter than is that of the ventricle. Moreover, in the atrium, there may be demonstrated the direct effects of vagus inhibition. Stimulation of the vagus nerve causes a shortening of the absolutely refractory period and a marked slowing of the recovery of excitability following a contraction. It is believed that considerations of functional changes in excitability and conduction should take into account not merely the changes in the absolutely refractory phase but also changes in the time course of the recovery from refractoriness. Whereas a normal heart will conduct an impulse from one chamber to another at a time certainly not much later than the end of the absolutely refractory period, it is obvious that in an inhibited or depressed preparation this is not the case. In such a preparation, it seems probable that time must be allowed for more or less complete recovery from relative refractoriness before conduction across a junctional region will occur. Furthermore, since the absolutely and relatively refractory periods may vary independently one from the other, a measurement of the absolutely refractory period is by no means a valid measure of the course of the relatively refractory period.

SUMMARY

1. Double condenser stimuli have been used for determination of the refractory period curves of the turtle ventricle and atrium.

2. Although the time to maximum of the stimuli used was in most experiments 0.018 second, this time may be varied over a considerable range without changing the form of the excitability curves plotted. Absolute values may be changed by such change of shock form.

3. For either ventricle or atrium of the normally beating heart, excitability as determined by such stimuli begins to rise at the end of the period

of absolute refractoriness, continues to rise without intermission, and is still rising at the time of the beginning of the next normal beat.

4. Under vagus inhibition there is a shortening of the absolutely refractory phase of the atrium. However, the relatively refractory period is prolonged and recovery to normal threshold level may be delayed indefinitely.

5. Vagus stimulation was not in any experiment observed to produce any direct effect upon the excitability of the ventricle.

6. Attention is called to the importance of considering the relatively as well as the absolutely refractory phase in studies of functional cardiac changes.

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THE INFLUENCE OF HYPERPNEA AND OF VARIATIONS OF THE O₂- AND CO₂-TENSION IN THE INSPIRED AIR UPON AFTER-IMAGES

ERNST GELLHORN AND IRWIN G. SPIESMAN

From the Department of Physiology, College of Medicine, University of Illinois, Chicago.

Received for publication April 17, 1935

In a preceding paper it has been shown that variations in the O₂- and CO₂-tension in the inhaled air brought about significant changes in hearing, which were attributed to alterations in the cortex of the brain. It seemed advisable to investigate whether similar changes could be obtained in experiments involving another sense organ and, therefore, a different cortical mechanism. For this reason the present study was undertaken in which the influence of CO₂ and O₂ was studied on a visual process. There are a few reports in the literature dealing with the effect of O₂-lack on vision. Most of them are incidental observations made during expeditions on high mountains (Hingston, 1925; Hartmann, 1933; and Barcroft, 1925). Quantitative studies by Wilmer and Berens (1918) showed distinct effects of O₂-lack on the visual field at an oxygen pressure corresponding to an altitude of 20,000 feet. Similar, but not very convincing results, were obtained by Goldmann and Schubert (1933). Bagby (1921) did not observe any changes in visual acuity, but Schubert in 1934 observed a considerable decrease in the distinction threshold for brightness.

Johnson and Paschal (cited by McFarland) believe that the effects do not concern the sensory mechanism, but are due to lack of "attention." There are no reports in the literature concerning the effect on vision of CO₂-excess or CO₂-lack, as is obtained in experiments with voluntary hyperpnea.

The influence of O₂-lack, CO₂-excess and hyperpnea was studied on after-images for two reasons: 1, they involve the fundamental visual mechanism; 2, they lend themselves to quantitative investigations (Gellhorn and Weidling, 1925; Gellhorn and Kühnlein, 1926) and appear, under suitable conditions, with a distinct and easily measurable latent period (Creed and Granit, 1928).

METHOD. The experiments to be reported in this paper were started after eight subjects had undergone a training period of several weeks, during which a very great consistency of the latent period of negative after-images was obtained when a colored square was fixated under stand-

ard conditions. The head was fixed in a head-holder, the eyes were closed for one minute prior to the fixation. The after-images were observed with closed eyes, and the latent periods measured with a stop-watch. The fixation was in most experiments binocular and in some monocular, without influencing the results. In the majority of the experiments a colored square (7.5 cm.²) on a black and grey background was fixated at a distance of 60 cm. The illumination of the room was adjusted to the sensitivity of the experimental subject, so that latent periods were obtained which could easily be measured.

In a later series of experiments uncolored objects were chosen for after-image formation. A diaphragm of 10 cm. diameter forming the anterior wall of a wooden box which was illuminated from the inside was used as a pattern for after-images. The negative after-image consisted of a distinct black circle. O₂-lack and CO₂-excess were produced as reported in the first paper of this series. During the period of voluntary, maximal hyperpnea, which at the rate of 35 to 90 per minute lasted from two to six minutes, the respiratory minute volume was increased about 1200 per cent.

RESULTS. A total of 62 experiments were performed, each of them consisting of a series of after-images obtained at intervals of at least 10 minutes, since it was found in preliminary experiments that in a trained subject the latent period remains very constant, provided that the interval is not shorter than 10 minutes. Several control experiments preceded and followed the observations during O₂-lack, CO₂- excess, etc.

The results of experiments on the effect of O₂-lack indicate that the effect varied with the O₂-concentration. Practically no change resulted from the administration of N₂-air-mixture of 13 to 16 per cent O₂. Occasionally a "supernormal" period was observed at the beginning of the O₂-lack period, which was apparent in a slight shortening of the duration of the negative after-image. In the major part of this series O₂ concentrations varying between 9.2 and 11 per cent O₂ were chosen (table 1). It was found that invariably under the influence of these greater degrees of O₂-lack the latent period of the negative after-image increased. Sometimes no after-image whatever was formed.

It is necessary to mention precisely the more outstanding subjective phenomena observed during these experiments. Ordinarily in most subjects upon closing the eyes a positive after-image was observed followed by a short dark interval. Hereafter a negative after-image appeared which was blurred at first, but developed very rapidly into one with sharp edges corresponding to the pattern of the object. It was this moment which was chosen for recording the latent period of the after-images. Under the condition of O₂-lack several changes in the appearance in the after-images were noticed. During the fixation of the colored square or of the white diaphragm the experimental subjects had the impression that the color

observed was less saturated and that the white diaphragm looked "as if the lights had been dimmed." After closing the eyes, the positive after-image did not appear at once, as it did in the control experiments, but there was a measurable latent period, and the positive after-image was considerably blurred. When, ordinarily after a dark interval, the negative

TABLE 1

The influence of O₂-lack, CO₂, and hyperpnea on the latent period of negative after-images

NUMBER	NAME	O ₂ -CONCENTRATION	DURATION OF O ₂ -LACK	LATENT PERIOD		
				Control before O ₂ -lack	At the end of O ₂ -lack period	Control after O ₂ -lack
		<i>per cent</i>	<i>minutes</i>	<i>seconds</i>	<i>seconds</i>	<i>seconds</i>
1	O.	10.2	8	15.3	∞	15.0
2	G.	9.8	7	3.1	∞	3.3
3	Sp.	10.5	14.5	5.1	22.4	6.2
4	St.	10.6	10	7.6	24.0*	6.0
5	G.	10.1	7	6.5	∞	5.9

NUMBER	NAME	CO ₂ -CONCENTRATION	DURATION OF CO ₂ -LACK	LATENT PERIOD		
				Control before CO ₂	At the end of CO ₂ -period	Control after-CO ₂
		<i>per cent</i>	<i>minutes</i>	<i>seconds</i>	<i>seconds</i>	<i>seconds</i>
1	Sp.	5.9	5.5	6.3	13.2	7.0
2	St.	6.4	5.0	11.5	∞	10.5
3	G.	6.0	5.0	2.3	3.7	2.7
4	F.	4.4	5.5	7.2	19.2	6.5
5	O.	4.8	4.5	17.0	∞	21.0

NUMBER	NAME	HYPERPNEA		LATENT PERIOD		
		Duration	Frequency per minute	Control before hyperpnea	At the end of hyperpnea	Control after hyperpnea
		<i>minutes</i>		<i>seconds</i>	<i>seconds</i>	<i>seconds</i>
1	Sp.	3	78	6.8	10.0*	7.7
2	G.	5	75	3.0	7.8*	2.7
3	St.	6	66	10.8	∞	9.9
4	O.	4	68	11.5	∞	15.0
5	F.	3.0	88	8.4	14.0*	10.8

* Experiment carried out several minutes after the end of O₂-lack period and hyperpnea respectively.

after-image appeared after a prolonged latent period, it was less saturated, of diminished intensity and duration. As mentioned above, it was found that under O₂-lack sometimes the after-images were so weak and blurred that they did not permit of a measurement of the latent period; moreover, not infrequently no negative after-images were formed.

As it is apparent from table 1, the effects were completely reversible, but it may be noted that a very considerable lengthening of the latent period was observed, even 10 or more minutes after the completion of the O₂-lack period (table 2).

Another series of experiments concerning the effect of CO₂-excess on after-images was performed. It was found that CO₂ in concentration below 4 per cent did not influence the appearance of after-images, although the respiration was greatly increased. If, however, concentrations above 4 per cent were administered, the after-images were altered in the same manner as was described following O₂-lack (table 1). The main difference between the two groups of experiments was a quantitative one, since the effects of O₂-lack were more pronounced than those of CO₂-excess. But even in this group distinct after-effects, that is, a lengthening of the

TABLE 2

TIME	LATENT PERIOD	TIME	LATENT PERIOD	TIME	LATENT PERIOD
minutes	seconds	minutes	seconds	minutes	seconds
1:55	13.5	11:08	11.0	10:13	6.2
2:06	13.0	11:18	11.7	10:27	6.4
2:18	13.0	11:28	12.0	10:44	6.6
2:23-2:49½	10.8 per cent O ₂	11:33-11:37½	7.0 per cent CO ₂	10:48-10:51	hyperpnea (rate 60 p.m.)
2:28	19.5	11:38	17.4	10:56	15.2
2:38	20.5	11:48	16.4	11:30	6.4
2:48	∞	11:58	12.4		
2:58	24.0	12:08	11.6		
3:08	12.4				
3:18	14.0				

latent periods, were occasionally observed after the end of the CO₂ period (table 2).

In a final series of experiments the influence of hyperpnea on after-images was studied. It was found that hyperpnea of one minute duration was ineffective in altering the after-images, while hyperpnea lasting from 2 to 5 minutes was regularly effective and led to a distinct increase in the latent period of negative after-images. The subjective phenomena (blurring; decrease in intensity) were similar to those described for O₂-lack. They occurred even at a time when the respiration was already restored to normal following a period of apnea (table 2).

The question arises as to whether the effects described are due to the influence of O₂ and CO₂ on the pupillary diameter, or concern the visual mechanism directly. For this reason experiments were performed in which only one eye was used and an artificial pupil was placed in front of the observing eye. The experiments indicate that even under these conditions

the same results are obtained. It may, therefore, be said that the influence of O_2 -lack, CO_2 -excess and hyperpnea on the after-images involve the visual mechanism itself.

DISCUSSION. From the description of these results, it is evident that there is a great similarity between our previous experiments on audition and those on visual after-images. It was shown that O_2 -lack, CO_2 -excess, and hyperpnea led to a diminution of auditory acuity. The experiments with after-images indicate that in all three conditions the visual mechanism is likewise impaired. The appearance of the negative after-images corresponds to that obtained in experiments with a weaker stimulus, since obviously the duration and the saturation of the after-images was diminished. However, it is not improbable that the results indicate not only a very considerable decrease in excitability of the visual mechanism, but also a qualitative change, since in control experiments variations in the intensity of the stimulus never led to any considerable lengthening of the latent period. In fact, in several experiments the latent period was constant for various intensities until on further reduction of the stimulus it became infinite since no after-image was formed (in agreement with Juhasz, 1920).

In order to study the possible importance of vascular changes on the latent period and appearance of after-images, the blood pressure was measured in a number of experiments. It was found that O_2 -lack did not alter the blood pressure in our experimental subjects, and the decrease of the blood pressure after hyperpnea amounted to not more than 6 mm. mercury and was very readily reversible. CO_2 led to increases of about 10 mm. mercury in systolic blood pressure. Control experiments in which, by means of physical exercise, the blood pressure was raised 20 mm. or more, show that blood pressure changes as such within the range observed in our experiments, have no influence on after-images.

Since O_2 -lack and CO_2 -excess cause vasodilatation, and since improved circulation would scarcely cause impaired function, the decrease in excitability which is apparent from the lengthening of the after-images must be due to chemical cellular changes, whereas the vasoconstriction accompanying hyperpnea and probably even outlasting it may fully explain the alterations in after-images described above. For a more complete discussion of the effect of hyperventilation the reader may consult the first paper of this series since the interpretation given there is equally applicable to the after-image experiments.

It should be mentioned that the results might be thought to be due to the inability of the experimental subjects to fixate properly. The long training makes this interpretation highly improbable. Moreover, control experiments in which the time of fixation was varied showed that with decreasing fixation time the latent period of negative after-images increases;

but it is very important to note that even the fixation during one second instead of 10, although it led to a lengthening of the latent period of the after-image, always caused a negative after-image, whereas, as reported in our experiments under the conditions of variations of the O_2 - and CO_2 -tension in the inspired air and after hyperpnea, the after-image was not infrequently absent. This indicates clearly that the effects of O_2 -lack, etc., are due to an alteration in the nervous mechanism involved.

We, therefore, come to the conclusion that alterations in the CO_2 - and O_2 -tension of the blood, as are obtained by inhalation of various gas mixtures and by hyperpnea, lead to a decrease in sensitivity of the visual and of the auditory mechanism. The effects of these various factors are quantitatively but not qualitatively different. The only conspicuous difference between the auditory and visual experiments lies in the fact that we have not seen a shortening in the latent period of the after-images and an increase in their intensity following O_2 -lack, CO_2 -excess or hyperpnea. In other words, there is no equivalent for the temporarily increased auditory acuity. We are, however, not inclined to stress this difference because of the temporary nature of the increased excitability which may have escaped us for the reason that we could not perform the experiments more often than at intervals of 10 minutes. Moreover, it may be mentioned that, according to Schubert (1934), after a decrease in the distinction threshold for brightness during O_2 -lack, a period of improved (above normal) brightness distinction was observed with readmission of air.

SUMMARY

The influence of O_2 -lack, CO_2 -excess and hyperpnea is studied in regard to the latent period and appearance of negative after-images. It is found that under suitable conditions (O_2 -concentration 9 to 11 per cent, CO_2 -concentration 4 to 7 per cent, or voluntary hyperpnea lasting for 2 to 5 minutes at a rate of 35 and more per minute) there is a lengthening of the latent period of negative after-images or even complete absence of any after-images. The effect may last as long as 12 minutes after the readmission of air. The results correspond to those obtained in a previous paper with audition and seem to point out that O_2 -lack, CO_2 -excess and reduction in CO_2 -tension of the blood due to hyperpnea each cause a lowered excitability of the fundamental nervous mechanism involved in vision.

The authors wish to acknowledge their indebtedness to Mr. L. F. M. Storm for his help in these experiments.

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THE PHASIC CHANGES IN CORONARY FLOW ESTABLISHED BY DIFFERENTIAL PRESSURE CURVES¹

HAROLD D. GREEN, DONALD D. GREGG AND CARL J. WIGGERS

*From the Department of Physiology, Western Reserve University Medical School,
Cleveland, Ohio*

Received for publication May 6, 1935

The question whether coronary flow under normal conditions is reduced or even stopped during systole, or, on the other hand, undergoes a marked acceleration, is still in dispute. Records obtained by flow-recorders of different design give quite different answers (Anrep and various associates, 1; Hochrein and associates, 2; Wiggers and Cotton, 3b; Gregg, 4). This divergence of opinion is occasioned by the fact that neither the theoretical principles according to which reliable instruments should be designed nor the tests by which their efficiency can be judged have been as clearly formulated as in the case of pressure recorders. Consequently their construction is guided too largely by empirical trials while judgment as to their efficiency remains essentially a matter of opinion.

Since consecutive changes in flow are the resultant of differences in pressure which exist from moment to moment in the peripheral and central ends of a coronary ramus and since the pressure variations in the central end closely follow those in the aorta (Wiggers and Cotton, 3a), the variations of flow through the intramural vessels of the heart should be determinable by the difference between aortic and peripheral coronary pressures recorded by calibrated optical manometers.

The limiting physical conditions for such deductions are *a*, that the peripheral pressure changes in coronary branches are essentially due to the effects of ventricular contraction and not significantly to communication of pressure from collateral vessels, and *b*, that peripheral coronary pressures (P.C.P.) can be recorded accurately *both* as regards contour and magnitude. In a previous paper (5) we presented evidence that transference of pressure from collateral channels is of no material importance in territory supplied by the ramus descendens anterior. Evidence was also presented that efficient optical manometers reproduce with accurate con-

¹ This investigation was aided by a grant from the Ella Sachs Plotz Foundation. Preliminary reports of this investigation were given before The National Academy of Sciences, in Cleveland, November, 1934, and The American Physiological Society, Detroit, April, 1935.

tour and time relations the peripheral pressure changes, but do not register the maximal systolic resistance which the myocardium is capable of exerting. Consequently it was necessary to devise expedients for determining the true maximum and minimum pressures with reasonable accuracy. Furthermore, since some investigators have questioned the validity of observations made upon hearts which are separated from their natural nervous control and perfused with liquids other than the unaltered blood of the animal, it is important that these determinations be made under conditions to which such criticisms do not apply.

PROCEDURE. The aortic pressure was recorded as previously by a calibrated optical manometer. The main branch of the ramus descendens anterior was exposed for a distance of about a centimeter by grasping the surrounding sheath with four mosquito forceps, incising it in a frontal line with a sharp knife and laying back the sheath. In this way the greater portion of the nerves accompanying the vessel are spared. An electromagnetic compressor was applied to the isolated ramus, so that the normal blood supply was maintained except for brief intervals of clamping described below. A second optical manometer was inserted into a side branch of the main coronary vessel just below the region of preparation.

The peripheral diastolic pressure and contour of the P.C.P. pulses were determined as in our previous publication (5), i.e., by recording pressures from a lateral branch of the ramus descendens anterior, while the main vessel was clamped for 4 or 5 beats. Unless the heart is very slow, stabilization does not occur for several beats after occlusion (fig. 1, A). Hence the diastolic pressure should not be read on the first beat. It is immaterial which subsequent one is selected as the diastolic pressures differ only by 2 or 3 mm. Hg. We have arbitrarily selected the pressure level just preceding the second of such beats.

In order to determine the true peripheral systolic pressure, lateral pressure curves were similarly recorded while the coronary artery was clamped centrally for brief intervals at a frequency slightly different from that of the heart beat. In this way fluid could be trapped in the coronary system at various pressures up to aortic systolic. The level at which such a closed coronary vessel just held pressure at a constant level during such clamping was taken as the critical value for maximum systolic peripheral pressure under the circulatory conditions existing at the time. While the procedure was similar to that used by Anrep and Saalfeld (1d) the data obtained served quite a different purpose.

The results of this procedure are illustrated in figure 1, B in which advancing periods of clamping (0.3 sec.) are marked in 5 beats. By considering these from right to left (1 → 5) their significance is better understood. In beat 1, clamping began late in diastole and extended well into systole. After an abrupt fall during diastole, the pressure rises during

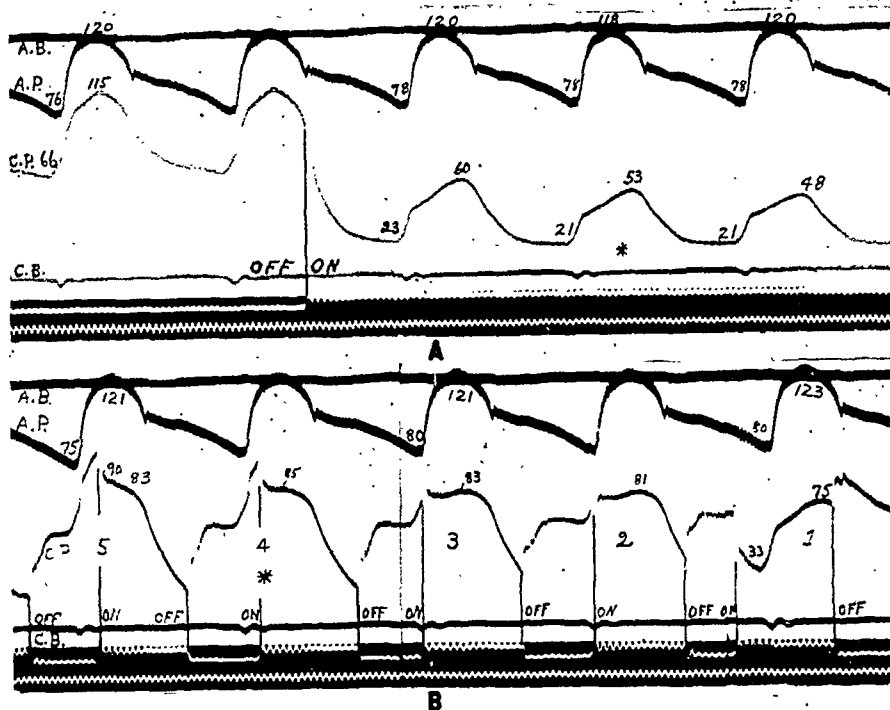


FIG. 1

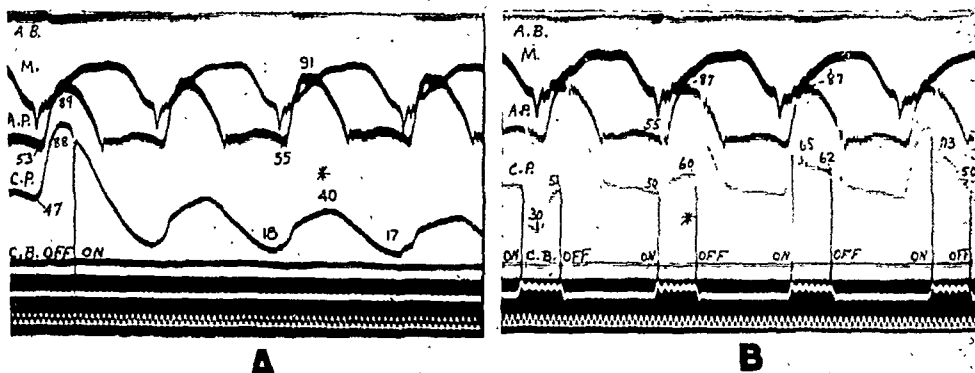


FIG. 2

Fig. 1. A, Record used for determining form of peripheral coronary pressure and the diastolic pressure. B, Record used for determining maximum systolic pressure by method of advancing short clampings. Description in text.

CP, coronary pressure; AP, aortic pressure; CB, coronary base line; AB, aortic base line. Periods of clamping in lower record denoted by "on" and "off." Time 0.02 second.

Fig. 2. Curves used for determining form and peripheral diastolic pressure (A) and systolic peripheral coronary pressures (B) under altered experimental conditions. Discussion in text. M, myogram; other lettering same as figure 1.

systole, reaching a height of 75 mm. The systolic part of the curve inscribed has the same form as that of a P.C.P. curve shown in figure 1, A. Although the beat develops from a relatively high diastolic pressure (33 mm. Hg) the systolic summit does not exceed 75 mm. Hg. In beats 2 and 3, clamping occurred during the normal systolic rise of coronary pressure. In both instances a slight additional rise to 81 mm. and 83 mm. respectively occurs. In beat 4, clamping took place after coronary pressure had exceeded such values. A pressure of 85 mm. is held during mid-systole. In beat 5 the vessel was clamped nearer the systolic peak at 90 mm., but from this level it gradually declined to 83 mm. at the end of systole. Such a series of systolic clampings definitely shows that the maximum coronary resistance expressed in terms of peripheral coronary pressure is less than 90 mm. and greater than 83 mm.; in fact, it is about 85 mm. Hg.

It may be added parenthetically that a survey of all our records obtained under a great variety of conditions has never revealed an instance in which such maximum resistance ever approximated aortic systolic determined from simultaneous aortic pressure curves.

Critique of method. The question arises whether values for systolic P.C.P. so established truly represent the full magnitude of the pressure developed in the intramural vessels of the left ventricle. Various sources of error were considered and tested. The possibility that leaks in the manometer system might account for the drop of pressures when vessels were clamped at the systolic pressure peak was checked in each experiment by clamping the vessel both centrally and peripherally to the side branch and in some cases by introduction of dyes into the whole system. The area of distribution of the ramus descendens anterior precluded the existence of branches to the auricle such as Anrep and Saalfeld encountered in using the circumflex ramus. That a "runoff" occurred by arterial anastomoses with the left ventricle or other arterial branches (coronary or extracardiac) is improbable since the "holding" pressure was less than that in other arterial branches or the ventricle. Indeed, in a previous communication (5) we analyzed particularly the reverse possibility, viz., that pressure transmission may occur from arterial collaterals, but we could find no evidence of significant functional anastomoses in normal hearts. To check the possibility that tributaries of the ramus descendens anterior to the right ventricle may play a part in reducing the actual maximal pressure, a heart was selected in which several large branches of ramus descendens anterior appeared to be directed toward the right ventricle. Clamping of all these branches was, however, without effect upon the form of the P.C.P. curve or upon the maximal peripheral systolic pressure established by the method of advancing short clampings. Therefore no other conclusion could be drawn than that the pressure exerted upon intramural

vessels is definitely less than that created in the left ventricular cavity. Such relationships are physically understandable. The ventricular walls during contraction cannot be regarded as a semi-liquid substance in which pressures spread equally in all directions, but are more comparable to a laminated elastic ball under internal pressure in which the tensions are unequally distributed. While the theory of distribution of elastic stresses in such globular structures has not been reduced to mathematical terms, sufficient is known to square our observations with the physical possibilities. At the time of maximal contraction, the tension at any point within the myocardium can be resolved into three vectors. Two of these have a direction perpendicular to equatorial planes at right angles to each other and the direction of the third is parallel to the radius of the circumference.

The tension (F per unit area) developed perpendicular to either equatorial plane is that which tends to separate the two halves of the spherical shell containing fluid under pressure. It is presumably equal at every point and may be roughly calculated by the formula

$$F = \frac{PR^2}{R_1^2 - R^2}$$

in which P is the tension per unit area exerted upon the internal surface, R is the radius of the cavity of the sphere and R_1 is the radius to the outside wall. Assuming a reasonable capacity and thickness of ventricular wall during contraction it can be calculated that the tension per unit area is less in either equatorial plane than that exerted upon the internal surface, i.e., than intraventricular pressure.

The magnitude of the third vector (parallel to the radius) is not the same at all points. It is greatest in the internal layers and becomes progressively less toward the exterior where it is zero. In other words, a gradient occurs from the interior to the exterior, much as would be the case if the pressures could be measured between numerous layers of a large series of thin rubber balloons inflated from the interior and jointly bearing the pressure. Consequently the mean tension developed parallel to the radius is necessarily less than that exerted upon the inner surface, i.e., than intraventricular pressure. Actually the distribution of elastic stresses is probably far more complicated and we merely offer this physical analysis as a tentative explanation of our experimental facts.

Differential pressure plots. It should be apparent that two such records as are shown in figure 1, A and B, when taken in rapid succession supply all the data for construction of differential pressure curves. The reconstruction illustrated by curves of figure 3 was carried out as follows: The beat denoted by * in figure 1 A, and the corresponding aortic pressure curve were enlarged by optical projection upon cross-section millimeter paper

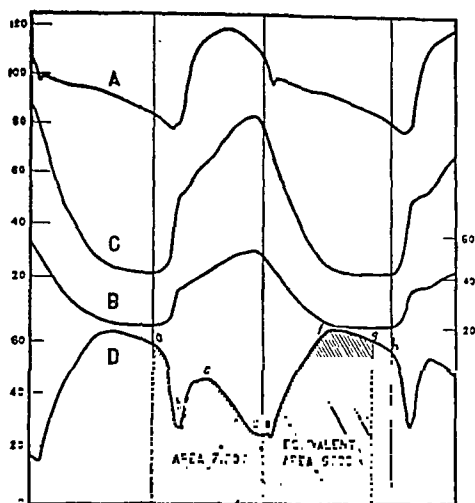


FIG. 3

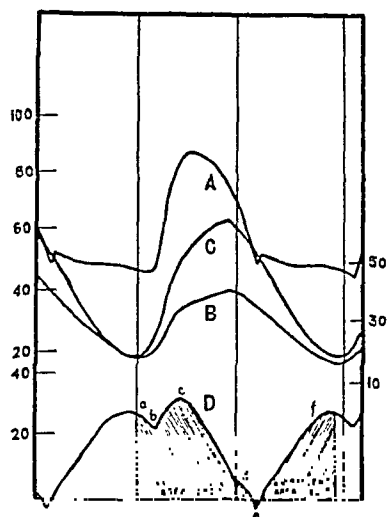


FIG. 4

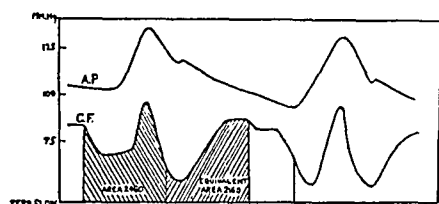


FIG. 5

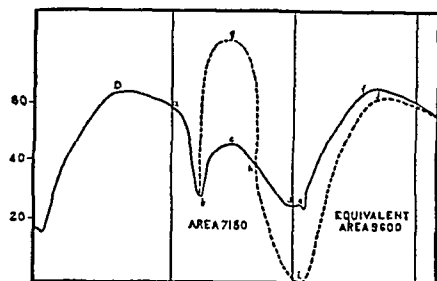


FIG. 6

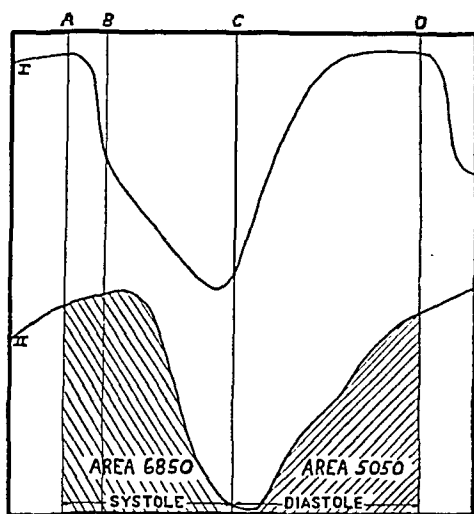


FIG. 7

Fig. 3. Reconstructions from curves of figure 1 showing relative volume flows and velocity in intramural coronary vessels during systole and diastole. A, aortic pressure; B, peripheral coronary pressure; C, same, reconstructed with true ordinate values; D, velocity curve. Shaded areas, volume flow with inscribed numbers indicating planimeter measurements of areas on original enlarged graphs. Ordinates, millimeter Hg; abscissa, time. Discussion in text.

Fig. 4. Reconstruction graphs from curves of figure 2; lettering same as in figure 3. Discussion in text.

Fig. 5. Reconstruction of flow curves from Anrep, Davis and Volhard 1c, figure 8, arranged to read from left to right. Discussion in text. AP, aortic pressure; CF, coronary flow.

Fig. 6. Comparison of intramural coronary velocities (curve D-a-c-e-f) redrawn from figure 3 and possible inflow velocities into coronary artery (curve b-g-i-j). Discussion in text.

Fig. 7. Comparison of coronary intramural flow curves when artery is perfused under constant pressure. Curve I, our curve determined from curve C, figure 3; curve II, curve of Anrep, et al., 1a, fig. 4. Both curves redrawn to same coordinate scales. A-B, isometric contraction; B-C, ejection; C-D, diastole.

and redrawn. The P.C.P. curve recorded by a calibrated manometer, varying slightly in sensitivity from the aortic manometer, was first brought to the same scale as the aortic and then the true P.C.P. curve was constructed by increasing the ordinate values of the whole curve so as to bring the maximum pressure to the systolic level established in figure 1 B (85 mm. Hg). In actual practice the two steps were combined in one operation and are illustrated by curve C of figure 3.

The pressure differences between the aortic pressure curve (A) and the true peripheral resistance curve (C), when again plotted with respect to zero yield curve D. We consider this to represent the coronary velocity curve. It should be emphasized that it actually depicts the *velocity of the "runoff"* from the coronary arterial system and does not correspond in every detail with the *velocity of inflow* into the coronary orifice which is affected in addition by changes in capacity as coronary pressures increase or decrease. Since sources for the "runoff" other than intramural capillaries seemed to have been excluded by our previous studies, such curves represent the velocity of intramural flow. By drawing vertical lines such as *a, e, g*, to the zero base line and measuring with a planimeter the areas so bounded, values are obtained which, when compared, express reasonably well the *relative volumes* flowing during different periods of the heart cycle. Such comparisons are not quite exact owing to slight differences in the size of vessels in different phases of the cycle; but the error so incurred is within the range of accuracy in redrawing and plotting such curves.

The phasic variations of coronary flow under natural conditions. Curve D of figure 3 is submitted as exemplifying changes of flow through the ramus descendens anterior under natural conditions. The contour of the aortic pressure pulses and the ordinate values for diastolic and systolic pressure are within normal ranges. Unfortunately the heart rate in our experiments was generally higher than we wished—in this case 107 per minute.

An analysis of the velocity curve (D) shows that coronary flow is sharply reduced during isometric contraction (*a-b*), increases sharply during the early half of systolic ejection (*b-c*), reaches a maximum with the summit of aortic pressure (*c*) and then decreases considerably during the latter half of systolic ejection (*d*). During isometric relaxation the flow accelerates, reaching a maximum (*f*) in mid-diastole despite the gradual decline of aortic pressure. Following this the flow remains large but decreases gradually; in fact it essentially follows the diastolic pressure gradient. Grossly described, two distinct accelerations occur: a first (*c*) during systolic ejection, and a second (*f*) during diastole. The positive wave representing the first acceleration is situated in a systolic valley and the maximum velocity (*c*) is less than that at any diastolic point subsequent to isometric relaxation. Such relationships indicate that velocity of coronary flow is definitely decreased by contraction; but it is never zero.

Measurement by a planimeter of the areas under curve D representing systole ($a-d$) and diastole ($e-h$) yields an S/D ratio of $\frac{7180}{11460}$ or $\frac{1}{1.6}$. Similar comparison of the systolic area with an equivalent area of early diastole yields an S/D ratio of $\frac{7180}{9600}$ or $\frac{1}{1.34}$, thus indicating that the *volume flow* during systole is 75 per cent of that during an equal time interval of early diastole. It has generally been recognized that an increase in coronary peripheral resistance during systole accounts for the decreased systolic volume flow but a pressure curve such as is illustrated by curve C in figure 3 emphasizes the additional fact that the resistance decreases about as gradually during diastole as it increases during systole. In this way gradually diminishing resistance is offered to flow until resistance reaches a diastolic level. In other words, contraction operates to decrease flow during the early portion of diastole as well as during systole.

By methods just described it has been found that similar phasic variation and relative magnitudes of volume flow during systole and diastole also occur in the left circumflex ramus. Thus in one experiment the ratio of flow during systole and an equivalent portion of early diastole was $\frac{1}{1.35}$.

The phasic relations under altered dynamic conditions. Thus far we have emphasized that under essentially normal circulatory conditions the systolic volume flow approximates but never quite equals that in early diastole. In 1931 Hochrein (2) advanced the conception that the maximal flow may shift variously between systole and diastole under different circulatory conditions. While not subscribing to his experimental proof, our analyses indicate that the flow might be maximal in systole under circulatory conditions in which diastolic pressure is relatively low and pulse pressure large. Experimental proof that this can occur is offered in the curves reproduced in figure 2 and their reconstruction in figure 4. The differential pressure curve (D) obtained shows that the first systolic wave, c , reaches a higher peak than the subsequent diastolic maximum at f . Measurement of equivalent areas gives an S/D ratio of $\frac{2320}{1215}$ or $\frac{1}{0.52}$, i.e., the systolic volume flow is almost twice as large as the diastolic. Such experiments indicate that our current conceptions must be revised by admitting that dynamic conditions *can be created experimentally* in which the maximum volume flow shifts from diastole to systole.

DISCUSSION. Our present analyses of flow through the intramural vessels under normal conditions depicted in figure 3 accord with observations of Wiggers and Cotton (3a) that lateral pressure curves give no evidence of decrease in coronary flow except during the period of isometric contraction when an extra preliminary oscillation occurs. The flow curves pre-

dicted by Wiggers and Cotton (3b) as a result of flow measurements under declining pressures also compare quite favorably. Even the sharp early systolic decrease in velocity missed by all previous observers was predicted. The investigators failed however to recognize the abruptness and extent of the systolic acceleration of flow and also the extent of its decrease during late systole.

Our interpretations of the systolic and diastolic coronary flow under natural conditions agree in part with those of Anrep and his associates; in several major particulars however our views are different. In our opinion these differences are partly attributable to use of different methods but many are merely a matter of interpretation. The cause of these differences, real and apparent, must be discussed, for no research is complete unless an effort is made to harmonize discrepancies. We are in fundamental agreement with Anrep and his colleagues 1, that ventricular contraction increases intramural resistance; 2, that *normally* the coronary volume flow is not greater during systole than diastole as claimed by German investigators, and 3, that, consequently, the beat of the ventricle mechanically reduces the minute volume flowing through the coronary vessels under otherwise identical conditions.

We feel however that the magnitude of the systolic reduction in flow was greatly over-estimated. In the first place distinctions between velocity and volume flow must be made. Our own curves show distinctly an initial sharp decrease in velocity followed by a subsequent increase which never attains the maximum found during late diastole. But despite these fluctuations, measurement of the areas beneath the curves shows that the volume flow during systole is still about 75 per cent of that during an equivalent interval of diastole.

If, as in figure 5, similar areas are measured under velocity curves published by Anrep, Davis and Volhard it is found that the *systolic inflow* into the coronary artery is even greater during systole than during an equivalent interval of diastole. Such curves are not quite comparable to our own, however. The systolic peak rises to a level higher than that of diastole owing to the fact that a component attributable to systolic expansion of the vessel is included. We cannot agree however that this systolic inflow volume is entirely determined by the elastic expansion of vessels. It is our impression that the clever experiments designed by these investigators to support such an argument may not have been as crucial as they believed. Thus they found that the systolic inflow is equal to normal even when coronary vessels are blocked by lycopodium. It must be recalled however that occluded vessels may accommodate more blood under the same systolic pressure in a main artery than when flow is unimpeded. This can easily be shown by placing a carotid artery in a plethysmograph and clamping the vessel peripherally.

An examination of the records published by Anrep, Davis and Volhard, one of which is redrawn in figure 5, gives, we believe, explicit evidence that a marked systolic intramural flow must have taken place. If the systolic part of flow curves were due wholly to capacity changes, then the curve should drop to a zero line at the point of maximum arterial expansion, i.e., at the peak of aortic pressure. *Since this is not the case*, but on the contrary the velocity never equals zero at any point, this must signify that some fluid was also moved through the intramural vessels during systole.

We have attempted to show by the dotted part of the curve in figure 6 the maximum extent to which our own curves of velocity might have been modified if the elastic accommodative changes during ejection had been added. Such superposition depicts at a glance how inflow velocities and outflow velocities of a coronary vessel may differ in phase without affecting the total volume flow per beat. This superimposed curve was obtained by assuming a flow of 0.3 cc. per beat and calculating the additional "probable increase" in capacity (determined from previously published volume-elasticity curves) at the peak of aortic pressure, then spreading the curves so that the area gained under *b-g-h* is subtracted by *h-i-j*. Such curves resemble the coronary inflow curves of Hochrein and associates even more than those of Anrep, Davis and Volhard. They emphasize how misleading interpretations of intramural flow based upon changes in velocity at a coronary orifice may become.

The flow at constant perfusion pressures. Since for technical reasons most of the studies on coronary flow require perfusion of the vessels with blood or oxygenated saline solution under a *constant* pressure and since Anrep and his associates are inclined to regard flow curves obtained under these conditions as most trustworthy it seems advisable to compare their curves with those predictable from curves of P.C.P. redrawn to a true ordinate scale. If in relation to curve C, figure 3, we assume a constant perfusion pressure above the maximum P.C.P. the flow curve should be represented by an inversion of this curve. Such a curve, together with a copy of the flow curve of Anrep et al. (1a) are redrawn in figure 7. The essential differences are 1, an earlier retardation of flow during systole, and 2, an earlier acceleration and a quicker return to a maximum in diastole in our curves. In brief, if the curve II were advanced 0.02 to 0.04 second it would show a general correspondence to our own. These differences, though apparently minor, are nevertheless important.

If we were likewise to determine the flow curves at progressively lower perfusion pressures from curve C, figure 3, it is obvious that the initial retardation would *always begin* with the isometric contraction and not progressively earlier as claimed by Anrep and Häusler (1b). Since we have shown that the early retardation of flow is due to a rise of myocardial

tension during isometric contraction it is difficult to conceive of any factual reason why the time of such retardation should be a function of the perfusion pressure. Anrep and Häusler's results are more probably due to progressing hypodynamic states associated with slower development of intraventricular and myocardial tensions combined with a natural lag of the flow-recording apparatus as a whole.

As regards the magnitude of the decrease in flow during systole when the vessels are perfused at constant pressures both curves of figure 7 show that the velocity of flow diminishes rapidly. According to our evidence systolic flow is never arrested, even momentarily, unless perfusion pressures are used which are below the maximal peripheral coronary pressures (e.g., 85 mm. Hg in the record under discussion). According to the curves of Anrep and his various associates complete arrest can occur momentarily somewhere toward the end of systole, even when perfusion pressures 50 mm. above aortic-systolic are employed. The fact that at most a momentary arrest occurs and that a considerable volume flow is nevertheless maintained is obvious when the areas determined by such curves are measured. As shown in figure 7, the systolic volume flow is even greater than during an equivalent period of early diastole (ratio 1.36:1.0). This fact which was most certainly evident to Anrep and his various associates was never strongly emphasized; on the contrary, readers of his articles who have no clear appreciation of the graphic evidence gain the impression that complete cessation of flow occurs for all or a large part of systole. Thus in their last article (1d) the phraseology again appears, "the blood supply to the heart *during systole* is negligible." It is regrettable that such unfortunate phraseologies have been repeatedly copied and transcribed by writers when in fact the actual records show that the volume flow during systole is as large as during early diastole when the vessels are perfused under constant pressures.

SUMMARY

Under experimental conditions which permit retention of a natural blood supply under pulsatile pressure and which spare the innervation of blood vessels, we recorded the form and magnitude of the central coronary pressure (aortic pressure) and by special expedients, detailed in the text, also the contour and magnitude of the peripheral coronary pressure. The differential pressure curve constructed from these quantitated records gives a *velocity* curve of intramural flow and the areas beneath different portions of such a curve with zero pressure as a base line allow comparison of the relative *volume flows* during various portions of the heart cycle.

Such curves show that under normal conditions the velocity of intramural flow is abruptly decreased during isometric contraction; the flow

accelerates while aortic pressure rises to its summit, again to be retarded during the last portion of systole. A great acceleration occurs during isometric relaxation and a maximum velocity is attained at about the time the ventricles begin to fill during diastole. During the remainder of diastole, the velocity gradually decreases, the degree of retardation at the end of the cycle, depending upon duration of diastole and the gradient of the drop in aortic pressure.

The changing resistance within the ventricular walls and the variations of aortic pressure during each cardiac cycle constitute continually opposing forces which determine the velocity of flow from moment to moment; but one or the other dominates at different times during each heart beat. The rapidly increasing resistance previous to the systolic elevation of aortic pressure accounts for the brusque retardation of early systole. Similarly the rapid decrease in myocardial resistance accounts for the prompt acceleration of diastole flow before ventricular filling begins. The secondary acceleration during systole as well as the continued high velocity during most of diastole are dominated by the aortic pressure.

A study of our curves shows that these fluctuations in *velocity of flow* during the cardiac cycle give no information regarding the *volume flow* during systole and equivalent periods of diastole. *Actual comparisons of such volume flows show that the myocardium receives approximately three-fourths as much blood during systole as during an equivalent interval of early diastole, but of course much less than during the entire period of diastole on account of its greater length.* Under exceptional experimental conditions characterized by relatively low diastolic pressures and large pulse pressures the systolic volume flow may even exceed that of equivalent periods in diastole.

Neither the phasic velocity nor volume flow *from* the larger coronary rami into and through the capillaries of the myocardium correspond exactly with the velocity or volume flow *into* the mouth of a coronary artery, for as Anrep, Davis and Volhard correctly emphasized the rate and volume of inflow are modified by changes in the capacity of the coronary vessels as the arterial pressure increases or decreases. It is therefore impossible as Hochrein and Keller have done to predict systolic and diastolic variations in intramural volume flow from velocity curves obtained at the mouth of an artery. The curves of figure 6 show how greatly the curves may differ. On the other hand, we have presented evidence of our own and point out suggestive evidence in curves published by Anrep, Davis and Volhard which indicates that the increased volume entering the coronary system during systole is not retained until it is moved onward during subsequent diastole; on the contrary a part of this systolic increase is moved forward through intramural capillaries during the period of systolic ejection.

An analysis of the changes in peripheral coronary resistance indicates that, contrary to general belief, the tension exerted by the myocardial contraction upon the intramural vessels is never as great as aortic systolic pressure. Consequently it is our belief that when coronary vessels are perfused under constant pressures, the flow is also not arrested—even momentarily—unless perfusion pressures very definitely below aortic-systolic are employed. Even in such curves, as in records presented by Anrep and his associates, the evidence is clear-cut that the *volume flow* during systole is quite as large as during an equivalent period of early diastole. *Physiologically it is more important to stress the maintenance of a good systolic volume-flow than the decreasing velocity and possible momentary arrest toward the end of systole, when vessels are perfused at constant pressures.*

Any interpretation or statement regarding coronary flow which implies that the myocardium receives only a negligible supply of blood during contraction—regardless of whether constant or pulsatile pressures are operating—is contrary to all hemodynamic evidence, that submitted by others as well as ourselves.

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THE PERIPHERAL MOTOR SYMPATHETIC INNERVATION TO AND WITHIN THE UTERUS¹

SAMUEL R. M. REYNOLDS AND SANFORD KAMINESTER²

From the Department of Physiology, Long Island College of Medicine, Brooklyn, N. Y.

Received for publication April 30, 1935

Several recent reviews attest to the fact that the peripheral innervation to the uterus and the nature of its intrinsic innervation are not understood (Davis, 1933; Gruber, 1934; Fontaine and Hermann, 1932). Indeed, Davis, who has summarized most of the contributions reported in the literature, concludes that "research by a considerable number of the most eminent authorities has failed to establish some of the most important details concerning the exact anatomy of the intrinsic nerve-supply of the uterus." It is generally agreed, however, that the uterus has within its walls an abundance of nervous tissue, richer about the utero-vaginal junction than elsewhere. Except for this region, the uterus of the rabbit is virtually devoid of intrinsic ganglia (Dr. A. A. Davis and Prof. W. P. Kennedy, personal communication). The morphological features of this intrinsic uterine innervation are totally unknown. Langley and Anderson (1895-96) showed many years ago that the motor sympathetic innervation to the uterus is through the vesico-uterine strands of the pelvic plexus, but the nerve pathways to the uterine cornua from this point have not been determined.

In the course of certain work relating especially to the hormonal control of uterine motility in transplanted uterine fistulae, it became desirable for us to know certain facts concerning the distribution of these nerve paths from the pelvic plexus to their respective, ultimate destinations. Accordingly, the following experiments have been performed. The experimental procedures adopted were based upon the possibility that in the rabbit nerves might pass from the vesico-uterine strands through the parametrium and send off fibers more or less locally to the uterus along the whole course of the parametrium.

PROCEDURES. The results reported below are based upon observations made on eleven mature, ovariectomized rabbits. In six of these, both uteri were used. Thus

¹ Aided by a grant from the Committee for Problems in Research of Sex, of the National Research Council.

² Member of the Department of Obstetrics and Gynecology.

the experimental procedures were carried out on seventeen uteri. The innervation to the cervical region was examined in eight of these. At every step in the following procedures uterine responses to presacral nerve stimulation were obtained repeatedly, two, three, or even more times.

The rabbits were anesthetized lightly with Dial (Ciba), 0.6 cc. per kgm. being given intraperitoneally. The uterus was exposed through a low midline incision and

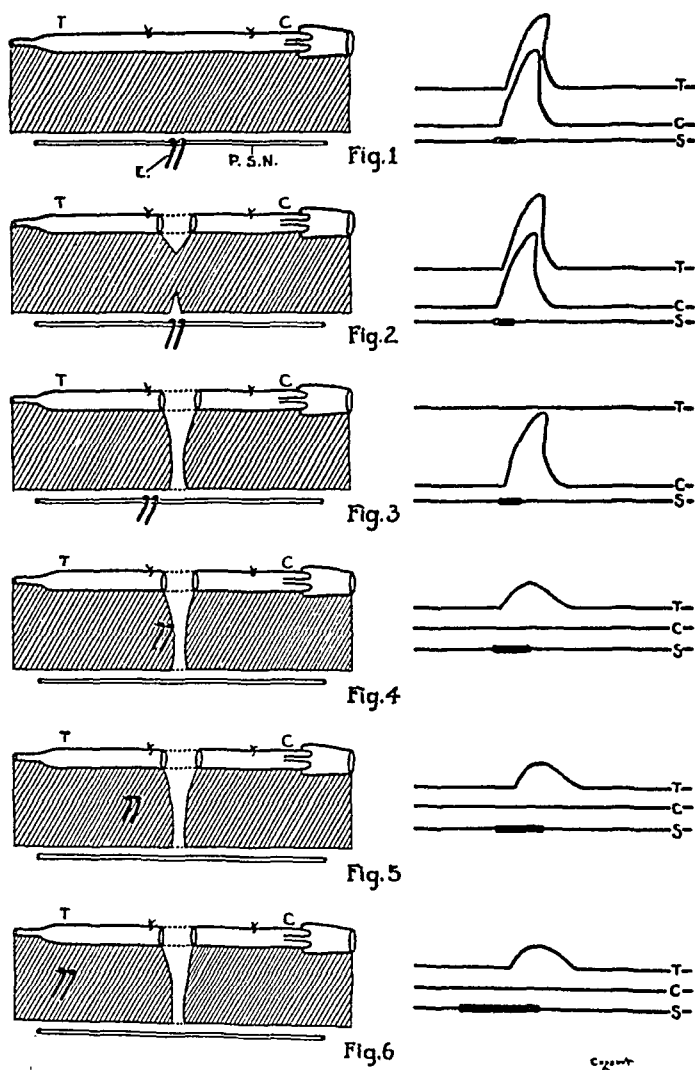
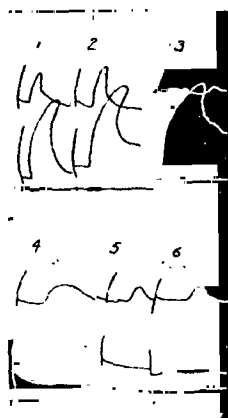


Plate I. Schematic representation of experimental steps performed (on left) and of results obtained (on right) in showing the non-essentiality of uterine continuity for responses of the whole organ to pre-sacral nerve stimulation. See text for discussion. Legend for this and other figures:

T, tubal end of uterine cornu to which thread for recording myographic activity is attached. C, cervical end of uterine cornu, to which a second thread is attached. E, stimulating electrodes. P. S. N., pre-sacral nerve. S, stimulation signal. ↑, epinephrine administered intravenously.

two threads, each connected with a long, light lever, were passed through the uterus. One of the threads was attached near the point of fusion of the two uterine cornua (C, plate I, no. 1), and the other, somewhat above the mid-portion of the uterus toward the tubal end (T, plate I, no. 1). The several strands of the pre-sacral nerve were carefully exposed about the level of L₅ and placed in a shielded electrode. Faradic stimulation of moderate strength was employed. Records of contractions from the two portions of the uterus were recorded in the manner shown in text figure 1. Time records were dispensed with since only the qualitative results were desired. In every case, however, the period of stimulation lasted for approximately 15 seconds.

After three to six initial uterine responses to presacral nerve excitation had been elicited, the parametrium and uterus were exposed in the mid-region between the points at which the threads were attached. The vascular pattern was studied and if, as sometimes happens, there was but one large vein draining the terminal end of the uterus and it lay across this region, it was spared as the parametrium was divided in the manner described below.



Text fig. 1. Excerpts from experiment on which diagrammatic results of plate I are based.

RESULTS. *Non-essentiality of uterine continuity for responses of the whole organ to pre-sacral nerve stimulation.* (Plate I; text fig. 1.) In this group of experiments the uterus was first semi-sected in the mid-region and cut completely through in the second step (plate I, no. 2). Following this, the parametrium was severed progressively in small steps of 5 to 10 mm. each. All such transections of the parametrium were made between two ligatures passed in appropriate steps at the level at which the tissue was to be divided. Between any two such successive steps the pre-sacral nerve was stimulated and uterine responses repeatedly obtained or attempted. As soon as a cut in the parametrium resulted in abolition of responses in the terminal, or tubal end of the uterus following stimulation of the pre-sacral nerve, electrodes were placed at this most recently cut portion of the upper parametrium and

responses of the terminal end of the uterus obtained (plate I, no. 4).

In this series of experiments, it was found that the uterus and approximately one-half to one centimeter of the adjacent parametrium could be transected *without* affecting responses of the upper segment of the uterus when pre-sacral nerve was stimulated (plate I, normal, no. 1; uterus severed, no. 2; text fig. 1, normal no. 1; uterus severed, no. 2). It was not until the parametrium had been cut through, usually in the second centimeter from the uterus (or approximately in the middle third of the parametrium) that a dissociation of responses occurred between the two segments of the uterine cornu when the responses were abolished in the upper but persisted in the lower segment (plate I, no. 3; text fig. 1, no. 3).

Demonstration that principal nerve paths lie wholly within the parametrium. (Plate II.) This point was investigated in the following manner. After

normal uterine responses to presacral nerve stimulation (plate II, no. 1) were obtained, the parametrium was divided progressively in small steps from the uterus to the retroperitoneal wall, or the transection was commenced at the retroperitoneal wall and continued toward the uterus. In still other experiments the transection was commenced in the middle of the parametrium, but *in every case the uterus, with a small bit of adjacent parametrium, was kept intact* throughout an experiment.

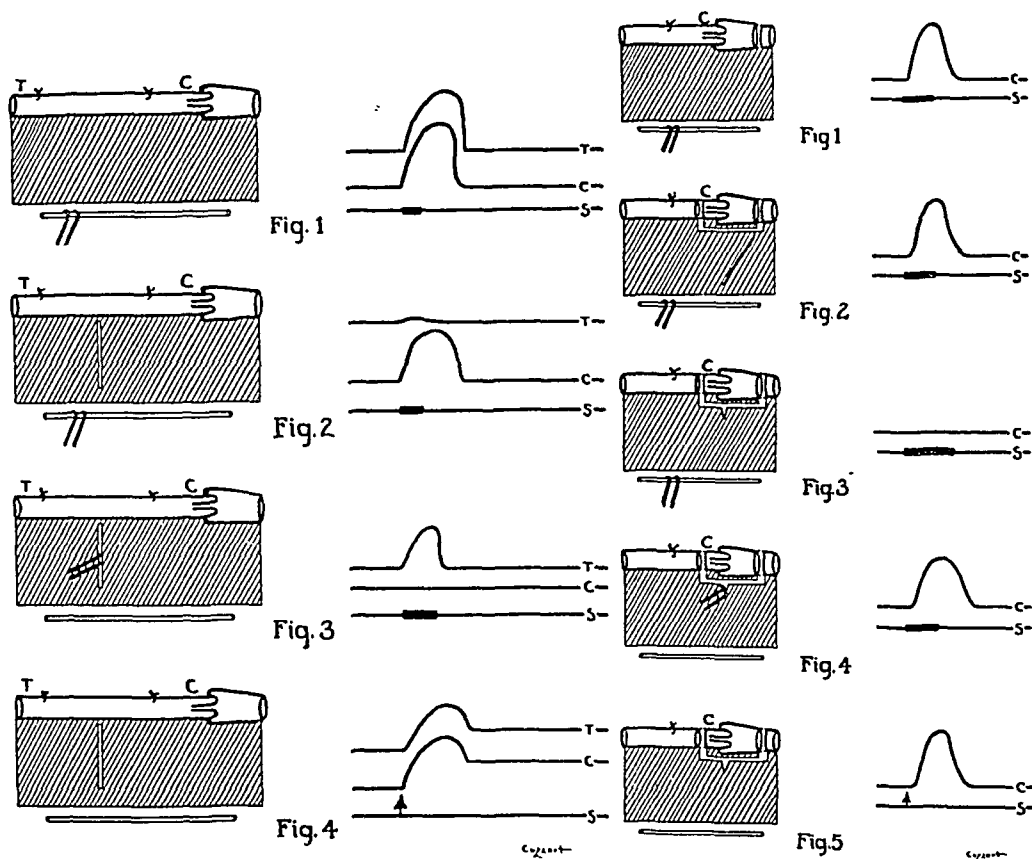


Plate II. Schematic representation of steps performed showing that *the main motor nerve paths to the whole uterus lie in the parametrium*. See text for description.

Plate III. Schematic representation of steps showing *the non-essentiality of the utero-vaginal junction (with ganglia uterini cervicalia) for nerve responses of the adjacent uterine cornua*.

In all of these experiments, a point on the parametrium was eventually reached, transection of which abolished responses of the terminal or tubal end of the uterus lying above the level of the cut (plate II, no. 2). This

point, as mentioned above, lies approximately midway between the retroperitoneal wall and the uterus. When electrodes were placed at the distal cut edge of the parametrium at the site where the last transection abolished the responses of the terminal end of the uterus to pre-sacral nerve stimulation, a contraction of the corresponding end of the uterus was obtained (plate II, no. 3). In order to show in certain experiments that in spite of some slight congestion the circulation was still good, epinephrine was administered intravenously (plate II, no. 4). If, as the result of epinephrine injection, the tubal end of the uterus failed to contract, the previous records were discarded.

These experiments show, therefore, that uterine continuity is not essential for pre-sacral nerve responses of the uterus but the integrity of part of the parametrium is essential. Thus the main nerve paths connecting the upper and lower portions of the uterus lie wholly within the parametrium.

The local distribution of nerves to the uterus from the parametrium and the extent of the intramural plexus. This point was approached by several aspects of our experiments and in such a way that certain conclusive statements may be made regarding it. In the first place, it was pointed out above that transection of the parametrium alone, leaving the uterus itself uncut, effected in the uterus above the level of section a loss of responsiveness to pre-sacral nerve stimulation. Thus we observe that one portion of the uterus is quiescent while another part, in structural continuity with it, contracts. Since Langley and Anderson showed that the sympathetic nerves to the uterus of the rabbit contain no inhibitory fibers,³ and since in these experiments uterine continuity is unimpaired, our results show that there is no intra-uterine innervation (plexus) capable of bridging the gap made by cutting the parametrium alone. It must be mentioned, however, that a delayed response in the upper portion of the uterus may occur if the ovariectomized rabbit has been treated with oestrin, in which case the uterus is very irritable. Such delayed contractions are due to propagation of visible, slow waves of muscular contraction, *and in this respect differ from the simultaneous contraction of the whole organ* that one observes if the pre-sacral nerve is stimulated when the parametrium is intact.

Other of our data are even more convincing, however, in showing that the local nerve supply to the uterus is by collateral branches from the main parametrial nerves. In certain experiments (in which the rabbits

³ Actually the responses one obtains to hypogastric nerve stimulation will depend upon the species and sexual state at the time of stimulation (Sauer, Jett-Jackson, Reynolds, 1935). The argument is not changed, however, since the uterine fibers are not mixed, i.e., excitatory *and* inhibitory.

have not been ovariectomized too long) one may obtain responses of the uterus by stimulating the parametrium directly. When this is done, the records one obtains are usually slow to reach a peak and to relax. The duration of the latent period in such experiments is determined by the relation of the point of stimulation to the point (thread attachment) from which the myogram is obtained. This is exemplified in the figures shown (plate I, nos. 4, 5, 6: text fig. 1, nos. 4, 5, 6). Here it may be seen that the latent period of no. 5 is double that of no. 4, and that of no. 6 is over four times as long as no. 4. The position of the electrodes in each case is indicated in the corresponding figures of plate I. In experiments such as these, it was observed that the uterine contractions commenced approximately opposite the parametrial site of stimulation, and from this position the wave of blanching and constriction spread slowly in both directions. It thus appears that the effect of parametrial stimulation is to initiate a local contraction which spreads by muscular conduction. This effect is seen to the best advantage when the stimulation is applied at the uppermost end of the parametrium, and differs markedly from the nearly simultaneous contraction of the whole uterus which results from stimulation of the pre-sacral or a main parametrial nerve. *The inference is clear, therefore, on the basis of these observations, that the motor sympathetic nerve fibers that enter the uterus from the parametrium do not innervate distant parts of the organ, but supply it only locally.*

In further support of this conclusion it should be mentioned that in those rabbits which have been castrated for some time, the local uterine contraction resulting from stimulation of the most distal end of the parametrium may be definitely seen, but the contraction so initiated dies out before reaching the site from which the records are obtained. Thus failure to record a contraction with this method of study is not to be construed as a negative result unless the whole uterus has been observed. Moreover, animals with oestrin, whether from their own ovaries or as a result of injection, are not suitable for these studies since the uterus is so irritable that spontaneous movements interfere with the observations. Still another factor to be taken into consideration is that the effects described above are to be seen advantageously only in rabbits whose uteri are long. With short cornua, distinctions in latent period of response to parametrial stimulation at selected sites are difficult to discern.

The non-essentiality of the utero-vaginal region for nerve responses of the adjacent uterus. (Plate III.) The relationship of the richly innervated utero-vaginal junction to the immediately adjacent uterine cornua was investigated. This was done to ascertain the essentiality or non-essentiality of this tissue for the uterine responses to pre-sacral nerve stimulation. Records of the lower uterine segment (C) were obtained under the three

following conditions: first, when the vagina was transected (plate III, no. 1); next, when the utero-vaginal junction was completely and carefully excised (plate III, no. 2), and finally, when a small incision was made in the parametrium at the level of the cervix (plate III, no. 3).

The results of these procedures showed that transection of the vagina does not abolish responses of the uterine segment just above the utero-vaginal junction, nor does careful excision of this whole region affect the nerve responses of the cornua (plate III, nos. 1 and 2). *Thus these data show that the principal nerves to the uterus do not go by way of the ganglia uterini cervicalia, since the tissue containing these structures may be removed without abolishing pre-sacral nerve responses in the uterine cornua.*

If one now makes a small cut approximately 2 to 3 mm. long at the margin where the utero-vaginal tissue was excised and at the level of the cervix prior to its removal, it is found that pre-sacral nerve stimulation now fails to elicit a response (plate III, no. 3). Stimulation of the peripheral cut margin at this spot will, however, elicit responses (plate III, no. 4). This point, where a small cut severs the main nerves to the uterus, is the approximate place indicated by Langley and Anderson at which they terminated their study of uterine innervation (Langley and Anderson, 1896, their diagram on p. 389). If one holds this portion of the parametrium to the light, he may see grossly these principal nerves which come from the pelvic plexus. The present observations testify, then, to the non-essentiality of the uterine cervical region and the adjacent vagina for uterine responses to pre-sacral nerve stimulation, but the main uterine nerves do pass very close to it.

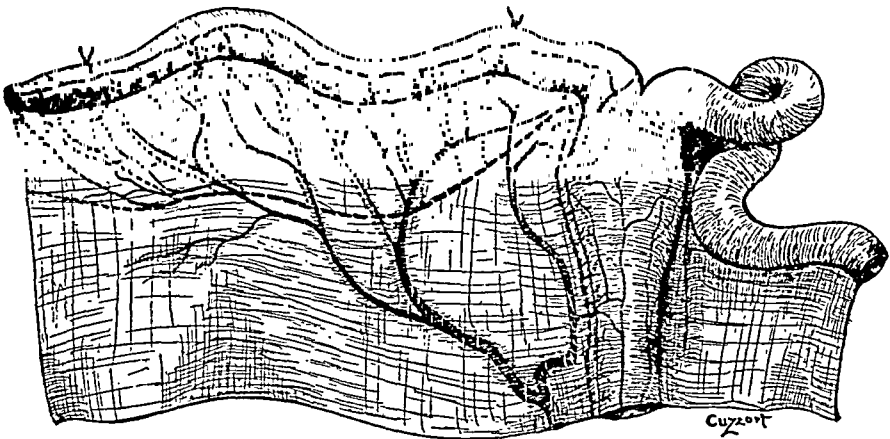
(It should be mentioned that the amplitude of the recorded contractions may diminish considerably upon transection of the vagina. If one sews the cut edges together the pull of the lower vagina is again exerted, but the nervous connection between the two portions has been severed. Such precautions must be exercised throughout work of this sort, else one may read into the results physiological inferences which are not warranted.)

CONCLUSION

The foregoing account forces us to adopt a definite picture of the peripheral motor sympathetic innervation to and within the uterus of the rabbit. This we have represented in text figure 2. The vesico-uterine strands from the pelvic plexus give rise to nerve fibers that pass close to the utero-vaginal junction and then dip more or less deeply into the parametrium as they run cephalad. Along their whole course they give off fibers which pass to the uterus. These collateral fibers innervate rather restricted regions of the uterus and do not contribute to a widespread, diffuse plexus within the uterine walls. This also applies to the highly nervous utero-

vaginal and uterine cervical portion of the genital tract, despite the presence of the uterine cervical ganglia.⁴

Finally, it should be mentioned that we have confirmed the observations of Langley and Anderson, that in addition to the motor fibers, vaso-constrictor fibers in the uterus are also stimulated upon excitation of the pre-sacral nerve. We have also observed in chronic experiments on cervicectomized rabbits that pallor (a mottling) of the uterus may occur when the pre-sacral nerve is stimulated, without myometrial contraction after



Text fig. 2. A uterine cornu showing the parametrium, the larger blood vessels and, by dotted lines, the general position in which the main motor sympathetic nerves to the uterus are found. When the uterus is short the nerves lie somewhat closer to the uterus than indicated here. See text for discussion of the motor nerves, the vasomotor nerves and of the intrinsic innervation of the uterus as well.

the main motor fibers to the uterus have been severed. Thus the vasomotor innervation to the rabbit uterus reaches this organ by paths other than in association with the peripheral motor innervation. Admittedly without proof, we tentatively suggest that the vaso-constrictor fibers to the uterus pass to their ultimate destination in close association with the vessels they innervate and which in this work remain intact.

⁴ It should be noted in passing that Langley and Anderson found that stimulation of the dorsal-most vesico-uterine strand causes contraction of the terminal half to two-thirds of the uterus; stimulation of the middle vesico-uterine strand causes contraction of the lower third to half of the uterus, and excitation of the ventral-most strand causes contraction of the lower vagina. We have made no attempt to confirm this observation, and for that reason have chosen to represent in text figure 2 the parametrial nerves as a dotted line to indicate the principal course of the many nerves in this region, rather than their number or actual distribution.

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THE INFLUENCE OF PROGRESSIVE TOXEMIC LIVER DAMAGE UPON THE DEXTROSE TOLERANCE CURVE¹

SAMUEL SOSKIN AND I. ARTHUR MIRSKY

From the Metabolic Laboratory of the Department of Physiology, Michael Reese Hospital,² and the Department of Physiology, University of Chicago

Received for publication May 9, 1935

Previous work has shown that the pancreas is not essential to the metabolic reactions determining the normal dextrose tolerance curve, while the presence of the normal liver is essential (1) (2). Our results indicated that the normal liver responds to an increased blood sugar, resulting from sugar administration, by decreasing its output of sugar which it was previously supplying to the blood from its own resources. That is to say, the administered sugar acts as an inhibitory stimulus to the liver so that the exogenous sugar supply temporarily replaces the normal endogenous supply, thus leading to the characteristically rapid disposal of the extra sugar and the return of the blood sugar to the normal level. This homeostatic liver mechanism has recently been confirmed by Tsai and Yi (3) who quantitated the sugar entering and leaving the liver by means of a flowmeter.

It has also been demonstrated that toxemia, which renders the dextrose tolerance curve abnormal in the intact animal, produces the same results in the complete absence of the pancreas under our experimental conditions (4). It was concluded that the abnormal dextrose tolerance curves occurring in toxemia are due to the effects of the toxin upon the liver and not the pancreas, and that the toxin interferes with the homeostatic liver mechanism which we have described.

The abnormal dextrose tolerance curves obtained in our previous toxin studies were all "diabetic" in character. We used large intravenous doses of diphtheria toxin in order to produce rapid and pronounced toxic effects. It has been shown, however, by Althausen and others (5), that in less acute toxemias where there is a longer survival period, the "diabetic" type of curve may give way to the "supernormal" before death intervenes. Clinically this variation in the abnormal curve caused by liver damage has been described by Judd, Kepler and Rynearson (6), and it is well known that "diabetic," "supernormal" and even normal dextrose tolerance curves

¹ Presented before the American Physiological Society, April, 1935.

² Supported by the Max Pam Fund for Metabolic Research.

may be obtained in cases of liver injury, without apparent relation to the degree of liver damage as judged by clinical or pathologic criteria. Indeed, this lack of correlation has been reported by Mann as applying to other tests of liver function (7).

In view of our previous work, we therefore thought it desirable to perform repeated dextrose tolerance tests throughout a progressive liver damage caused by toxemia, in an attempt to correlate the variations of the abnormal curve with the homeostatic liver mechanism described above.

METHODS. All these experiments were performed on unanesthetized normal dogs which were trained to lie quietly on an animal board while the injections and blood sampling were done by means of venous puncture with hypodermic needles. Food was withheld for 18 hours prior to each experiment and the test sugar was always given intravenously.

As in the previous work, toxemia was produced by the intravenous injection of diphtheria toxin.³ Since we desired a more slowly progressive toxemia than in the previous experiments, we employed a dose of 4.8 minimum lethal doses per kilogram body weight, which had been found by preliminary experiments to result in death of the animal in about 10 hours.

After a preliminary control dextrose tolerance test, the toxin was administered and the tolerance curves repeated consecutively until exitus intervened. Three series of tests, using different amounts of test sugar, were performed. In the first series, 1.75 grams sugar per kilogram body weight was administered at intervals of 2 hours. In the second and third series, 0.9 and 0.25 gram sugar per kilogram body weight respectively were given at hourly intervals.

The course of repeated tolerance tests, with the above doses of sugar but without toxin administration, and the effects of the injection of the experimental dose of toxin without the tolerance tests, were observed in a number of control experiments.

RESULTS. The nature of these experiments necessitated the determination of so many blood sugar values that it is impractical to present our data in tabular form. With minor variations, the results of each type of experiment on a number of animals agreed within narrow limits. Typical results are graphically illustrated in figures 1 to 3, and are grouped so as to facilitate comparison.

Dextrose tolerance curves obtained with 1.75 grams dextrose per kilogram body weight. Figure 1 presents typical successive dextrose tolerance tests, with (—●—) and without (—●—) the administration of toxin, when the test dose of dextrose was 1.75 grams per kgm. body weight. The effect of the same dose of toxin without sugar administration (—○—)

³ We are indebted to the Eli Lilly Co. for a supply of standardized potent diphtheria toxin.

is also included. Toxin alone is followed by a small but sustained rise in the blood sugar level lasting for about 6 hours, at the end of which time the level begins to fall very slowly for another 2 hours. In the final 2 hours there is a precipitous fall of the blood sugar to hypoglycemic levels, accompanied by typical hypoglycemic convulsions. The nature of this final phase is such that it can only be ascribed to a sudden and complete failure of the glycogenic functions of the liver, the organ which has been shown to be the only endogenous source of blood sugar (8).

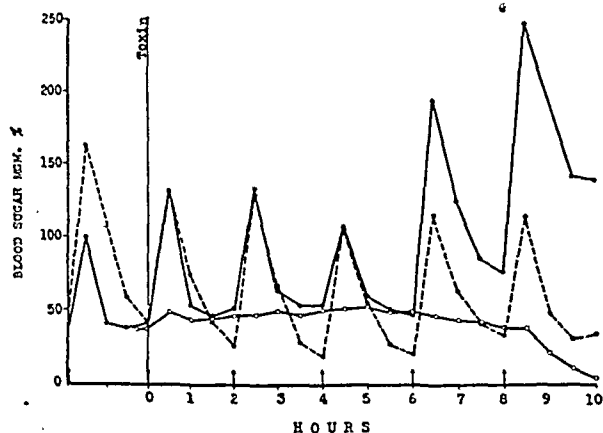


Fig. 1

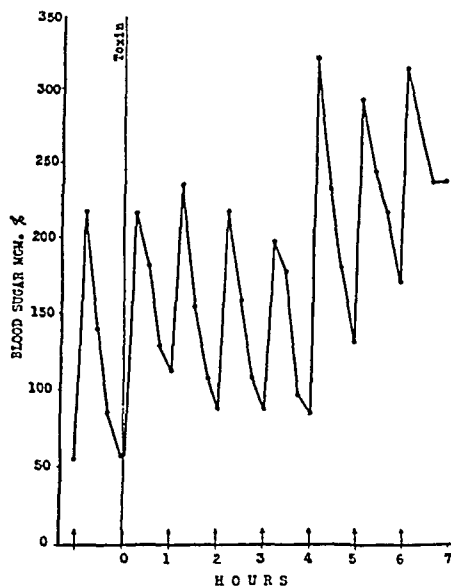


Fig. 2

Fig. 1. Successive dextrose tolerance curves, obtained with 1.75 grams dextrose per kgm. body weight, administered intravenously. Arrows represent sugar administration.

- Control series; no toxin given.
- Toxin given after single control curve.
- Effect of toxin alone; no sugar given.

Fig. 2. Successive dextrose tolerance curves, obtained with 0.9 gram dextrose per kilogram body weight, administered intravenously. The initial control curve is followed by toxin administration. Arrows represent sugar administration.

The control series of dextrose tolerance curves, when no toxin is given, shows progressively lower peaks and greater overswing below the initial blood sugar level, until the fourth curve is completed. The subsequent curves show a cessation of the increase in tolerance, although both curves remain within normal limits. We have evidence to show that such curves may be repeated for 24 hours and still remain within these same limits.

The dextrose tolerance curves obtained after toxin administration do not show the normal increase in tolerance, although they remain within

normal limits. The last two curves, however, which parallel in time the falling blood sugar level caused by toxin alone, show a sudden change to the "diabetic" type of response which continues until exitus supervenes. The failure of the earlier curves in this series to show the normal increase in tolerance might be interpreted as a slight "diabetic" tendency, but might also be ascribed to a masking effect of the rise in the basic blood sugar level caused by the toxin itself. The latter relationship, however, can not be postulated for the final two curves since they proceed in a direction opposite to the toxin curve. It seems clear that these final "diabetic" curves are related to the failing hepatic function in accord with our previous observations on the "diabetic" type of dextrose tolerance curve obtained with the same test dose of sugar in hepatectomized animals.

Dextrose tolerance curves obtained with 0.9 gram dextrose per kilogram body weight. Figure 2 presents a typical series of dextrose tolerance curves

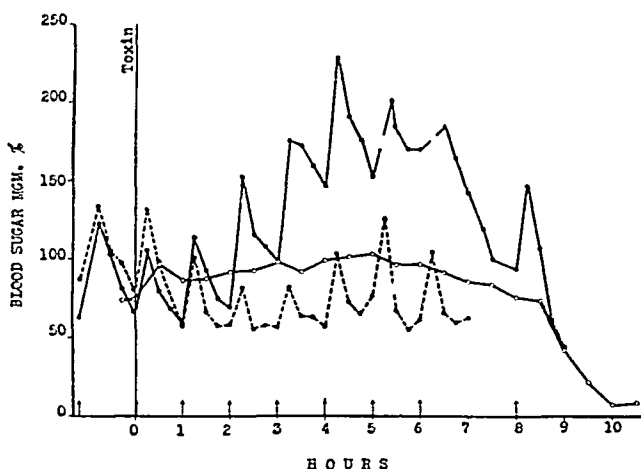


Fig. 3. Successive dextrose tolerance curves, obtained with 0.25 gram dextrose per kilogram body weight, administered intravenously. Conventions as in figure 1.

obtained after toxin administration, when the test dose of sugar was 0.9 gram per kgm. body weight. As the control test before toxin administration shows, it was possible to administer this smaller amount of sugar every hour and so obtain more frequent curves. It should be noted that the administration of the toxin causes an immediate "diabetic" type of change in the character of the subsequent tolerance curve, but that the repetition of the sugar dosage returns and holds the curves at a normal contour until the final "diabetic" sequence, coincident with liver failure, occurs.

Dextrose tolerance curves obtained with 0.25 gram dextrose per kilogram body weight. Figure 3 presents typical successive dextrose tolerance tests, with (—●—) and without (---●---) the administration of toxin, when the test dose of sugar was 0.25 gram per kgm. body weight. Again, as in figure 1, the effect of toxin alone (—○—) is included for comparison.

With this small dose of sugar, the "diabetic" nature of the second curve, after toxin is very apparent, and this type of curve persists until the fifth curve is reached when (as occurred much earlier with 0.9 gm. dose) the normal response reappears. However, here the resemblance to the previous series ends, for, instead of "diabetic" tolerance curves, "supernormal" curves are obtained during the period coinciding with hepatic failure.

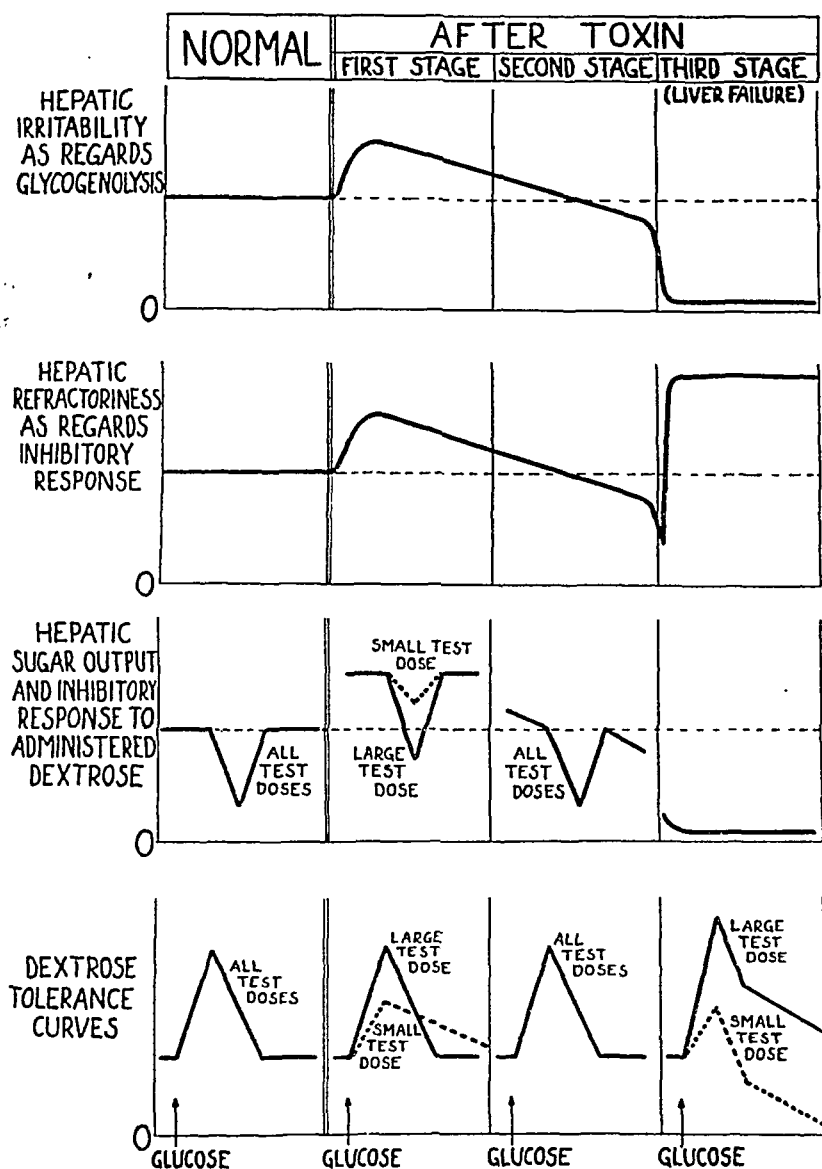


Fig. 4. Schematic representation of the progressive change in liver response to administered sugar, following toxin administration. It should be noted that the four items which are charted do not represent different processes, but are rather four different aspects of the same phenomenon.

In this connection it should be pointed out that in using a test dose of 0.25 gram glucose per kgm. body weight every hour, we were administering just about that amount of sugar which Mann and Magath (9) have shown can be disposed of by the completely hepatectomized dog.

SUMMARY AND DISCUSSION. A consideration of all the results obtained with the various doses of sugar used reveals 3 stages in the progressive change in the liver response to the administered sugar, following toxin administration; and indicates the important influence of the amount of sugar used for the test on the character of all 3 stages. In figure 4 we have attempted a schematic representation of these phenomena.

Stage 1 is characterized by an early decline in the inhibitory effect exerted by the administered sugar, so that the liver fails to decrease its own supply of blood sugar as rapidly as normal, and a "diabetic" type of tolerance curve results. This refractoriness of the liver to a stimulus to which it would normally respond is apparently related to the strength of the stimulus, since with larger doses of sugar the resistance may be overcome and normal curves obtained, while with smaller amounts of sugar the "diabetic" tendency becomes more and more apparent.

Stage 2 shows a return of the hepatic response towards the normal so that normal tolerance curves again appear. It is difficult to estimate how much of this effect, with a test dose of 0.25 gram sugar per kgm. (fig. 3), is due to an increasing responsiveness on the part of the liver and how much is due to a summation of inadequate stimuli, since the repeated administration of this small amount of sugar caused a steady rise in the basic blood sugar level during *stage 1* which may finally have represented a sufficient stimulus to the liver to cause it to give the normal inhibitory response, in spite of some remaining refractoriness. That *stage 2* is real and not an artefact, however, is apparent from experiments with larger amount of sugar, such as illustrated in figure 2, where the initial "diabetic" tolerance curve gives way to the normal, which persists until the final stage is reached, in spite of a falling base-line of blood sugar level.

Stage 3 consists of a more or less complete failure of hepatic function so that the effects obtained correspond closely to those observed in the liverless animal. Since the extrahepatic tissues can dispose of approximately 0.25 gram dextrose per kgm. per hour, the administration of less than this amount of sugar yields curves simulating a "supernormal" hepatic response, while the administration of greater amounts of sugar leads to an accumulation in the blood simulating a markedly "diabetic" type of curve.⁴

⁴ A more detailed study of all our data seems to indicate that, between this final stage and the preceding stage of normal response, there is a transitory phase in which the liver retains a large part of its function but reacts in an abnormal measure to sugar administration so that truly "supernormal" responses are obtained.

In a previous report we have referred to the work of others which indicates that the first effect of a poison on the liver is to act as an irritant to the glycogenolytic mechanisms. We pointed out that it seemed reasonable that such a hyperirritability of the liver cells as regards the pouring out of sugar into the blood should limit the extent to which a given rise in blood sugar would inhibit this process. As the effects of the toxin on the liver progress to the point of mortal damage to the hepatic cells, the latter must pass from the state of glycogenolytic hyperirritability, through normal irritability, to hypoirritability and death. Translated into terms of the inhibitory reaction which determines the character of the dextrose tolerance curve, this cycle of events would be: First, a decreased inhibition of glycogenolysis yielding "diabetic" tolerance curves, unless the strength of the stimulus as represented by the administered sugar be great enough to overcome the refractory state of the organ, when a normal inhibitory response and therefore a normal tolerance curve may be obtained. Second, a return to the normal inhibitory reaction yielding apparently normal curves. Third, an increased inhibition of glycogenolysis yielding "supernormal" curves. This corresponds to the transitory phase between stages 2 and 3 mentioned in the footnote on page 654. And finally, a cessation of sugar output from the liver yielding the effects obtained in the hepatectomized animal. Our present data are in accord with the above conception.

It is clear from our results that a supposedly normal dextrose tolerance curve may, under appropriate circumstances, represent a greater degree of liver damage than a "diabetic" curve, and lesser degree of injury than a "supernormal" curve. This may account for the unsatisfactory nature of the attempts, mentioned in our introductory remarks, to correlate the results of dextrose tolerance tests with the clinical or pathologic criteria of liver damage. Such curves can be more correctly interpreted in the light of the cycle of events which we have demonstrated, and in conjunction with other evidence as to the extent and duration of the hepatic impairment. It is evident that a series of dextrose tolerance tests, performed at intervals during the course of a hepatic impairment, should yield information of greater prognostic value than could possibly be derived from any single test.

It is also possible that a comparison of tolerance curves obtained with large and small doses of sugar might be of clinical value since in stage 1 the large dose yields more normal curves than does the small dose, while in stage 3 the reverse is true.

CONCLUSION

1. The sequence of dextrose tolerance curves obtained by repeated tests during a progressively increasing toxemic liver damage, is related to the

nature and degree of the interference with the homeostatic mechanism by which the liver normally decreases its output of blood sugar in response to the influx of exogenous sugar.

2. The cycle of events following the onset of the toxemia depends on the amount of sugar used for the test. It is not a progression from the normal to increasingly abnormal curve, but passes from the normal to the "diabetic" type, then back to the normal, and again to the abnormal which may now be either "supernormal" or markedly "diabetic."

3. The relation of these results to the interpretation of the dextrose tolerance test is briefly discussed.

We wish to express our appreciation for the technical assistance of Mr. Jack Tarshis.

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LYMPH FORMATION DURING GLANDULAR ACTIVITY

OCTA C. LEIGH

From the Department of Physiology, Harvard School of Public Health, Boston, Mass.

Received for publication May 11, 1935

In addition to the long recognized importance of motion as affecting the rate of lymph flow, many authors ascribe great importance to osmotic changes in the tissue spaces as a factor in the increase of lymph flow observed during periods of activity.

This concept is based upon the work by Asher and Barbèra (1898) who reported an increase in the rate of lymph flow from the cervical lymphatic vessels after stimulating the salivary glands by placing blotting paper soaked with vinegar upon the tongue of a dog. Bainbridge (1900-1901) elaborated the above work, and also found an increase in the rate of lymph flow when the submaxillary gland was stimulated electrically through the chorda tympani and cervical sympathetic nerves or by pilocarpine injections. He later (1902) explained this increase during periods of glandular activity as possibly the result of a rapid formation of crystalloid metabolic products in the active gland cells; these metabolites entered the lymph spaces, probably by diffusion, and raised the osmotic pressure of the lymph, thus attracting water. An increase in lymph flow was the result.

Carlson, Greer and Becht (1907), in ten carefully performed experiments upon the submaxillary gland of the dog, could not confirm the work of Bainbridge nor could they obtain more lymph when the parotid gland of the horse was secreting actively. They offered no explanation for their negative results and their work remains unquoted.

Barcroft and Kato (1915-1916) approached the problem indirectly. They made no attempt to measure lymph flow. From the rise of hemoglobin in the blood as it traversed the actively secreting submaxillary gland of the dog, they calculated the fluid exudate from the blood during periods of activity and suggested that fluid unaccounted for as saliva was lymph. They concluded that increased functional activity occasioned an increased lymph flow.

Similar studies (Bainbridge, 1905; Falloise, 1903; Wertheimer, 1906; and Schmidt and Hayman, 1929-1930) on the liver, pancreas and kidney have been reported with conflicting conclusions. Most of this work may be criticized on account of the quantity and nature of the substances injected into the blood stream to induce glandular activity.

In view of the many contradictions in the literature upon this important subject and the improved methods of anesthesia and observation now available, it was thought the work of Bainbridge on the submaxillary gland should be repeated.

EXPERIMENTAL. Dogs ranging from 16 to 30 kgm. were anesthetized with nembutal intraperitoneally. The cervical lymphatic vessels were exposed and one cannulated on each side of the neck. The lymph vessels that drain the submaxillary gland lead in varying numbers from the hilus and enter a large lymph node which is situated near the bifurcation of the common carotid artery. From this lymph node one or two large vessels pass downward, closely paralleling the common carotid arteries, to terminate by entering the thoracic duct on the left and the subclavian vein on the right. If two of these vessels are present they have several cross communications. It is reasonable to suppose that if one of these vessels is cannulated and the other ligated as they enter either the thoracic duct or subclavian vein, the cannulated vessel will deliver the lymph produced in the area massaged in the head and neck region.

The lymph was allowed to flow into pipettes of 2 mm. diameter calibrated to 0.01 cc. The pipettes were placed in a horizontal position to avoid pressure changes. A small amount of heparin was placed in each cannula to prevent clotting. The average minute flow was calculated from readings made every five minutes except in a few experiments when minute readings were made. In order to secure lymph flow, regular massage (two vigorous strokes per minute) has been applied over the submaxillary gland and down each side of the neck throughout these experiments.

The relation of lymph flow to lymph formation. There is no flow of lymph or but slight flow from the cervical vessels in the quiescent animal until massage or motion is applied to the head and neck region. It is simpler to show the relation of lymph formation in the massaged area if the results are compared with the findings reported recently (White, Field and Drinker, 1933; and Weech, Goettsch and Reeves, 1934) on the rates of lymph flow from the extremities of dogs.

When the figures presented by these authors are charted (fig. 1), it is observed that there is no lymph flow during periods of rest. When motion (walking) is begun in the normal dog there is an initial rapid increase in lymph flow which falls quickly to a lower level and is there maintained as long as walking is continued. A slight increase was observed when activity was increased (running) but this was small when compared with the initial rise.

Weech, Goettsch and Reeves show this even more strikingly in their studies on dogs rendered edematous by plasmapheresis or by low protein diet. They report, "With the edematous dog the situation is similar but because the interstitial spaces contain more fluid (edema) the initial rapid

flow can be maintained for a longer time than in the normal animal. Within 10 or 15 minutes, however, the rate of flow decreases and continued activity is accompanied by progressive and finally by complete loss of edema." When the lower constant level was reached it was only slightly higher in these abnormal animals than the values observed by White, Field and Drinker for normal animals.

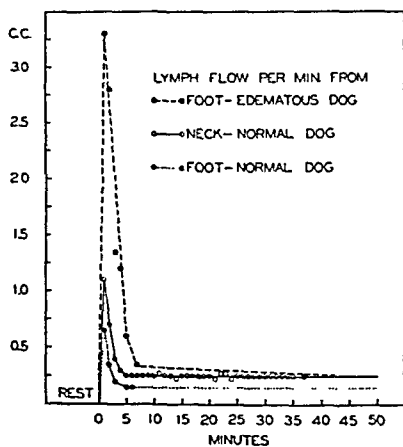


Fig. 1

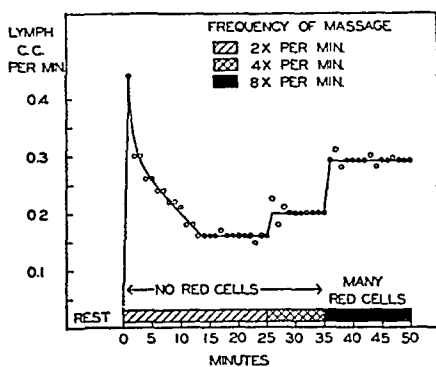


Fig. 2

Fig. 1. Lymph flow during walking or massage. Curve ●---● (foot—edematous dog), from Weech, Goettsch and Reeves, table 2. First minute flow calculated from first 42 seconds flow of table. Curve ●.....● (foot—normal dog), from White, Field and Drinker, table 4.

Fig. 2. Effect of increasing rates of massage over submaxillary gland on lymph flow from cervical vessels.

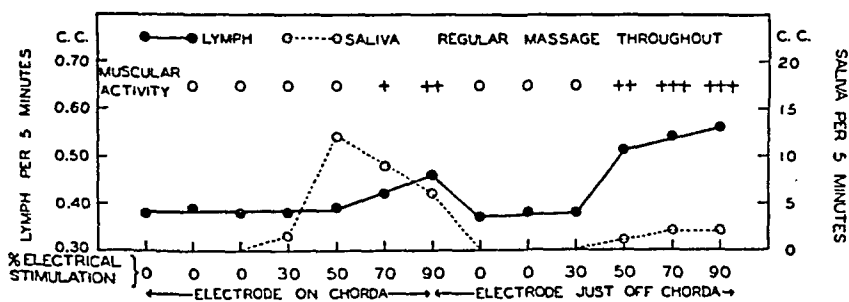


Fig. 3. Effect of increasing electrical stimulation on lymph and salivary flow.

When massage is applied over the submaxillary gland and down each side of the neck at regular intervals, the same typical rates of flow are observed from the cervical vessels. The variations that occur when the frequency of massage is increased is best shown in figure 2. Here again the increased massage, as increased activity, caused a rise in the lower constant level but it is small when compared with the initial rise. Very

frequent massage causes capillary damage, as is evident by the increase in the concentration of proteins and the appearance of erythrocytes in the lymph. But in our experiments, when too severe massage has been avoided, it is believed capillary permeability remained unaltered since the concentration of lymph proteins reached a low constant level simultaneously with the low constant level of lymph flow. Furthermore, no erythrocytes were observed in the lymph.

It appears obvious, as pointed out by Weech, Goettsch and Reeves, that the initial rapid rise in lymph flow represents the fluid of the interstitial spaces and the lower constant level is equivalent to capillary filtration or lymph formation. Activity, active or passive, supplies motion to force the tissue fluid and capillary filtrate into and along the lymph channels.

RESULTS. Table 1 shows the results of six experiments where electrical stimuli were used to activate the submaxillary gland. Lymph and salivary

TABLE 1

NUMBER OF EXPERIMENT	BEFORE SUBMAXILLARY ACTIVITY			DURING SUBMAXILLARY ACTIVITY			DURING SUBMAXILLARY ACTIVITY PLUS MUSCULAR CONTRACTIONS		
	Lymph flow	Salivary flow	Observation	Lymph flow	Salivary flow	Observation	Lymph flow	Salivary flow	Observation
	cc. per min.		min.	cc. per min.		min.	cc. per min.		min.
1	0.027	None	30	0.030	1.20	30			
2	0.026	None	40	0.023	1.30	30			
3	0.080	None	35	0.100	1.70	20			
4	0.101	None	45	0.080	1.07	10			
5	0.033	None	35	0.034	0.90	10	0.063	1.39	10
6	0.073	None	20	0.077	1.90	10	0.107	0.78	10
Averages...	0.051	None		0.057	1.34		0.850	1.08	

flow are recorded from one side only—that in which glandular activity was produced—since the opposite (control) side showed no variation from the normal. The only suggestion of an increase in the rate of lymph flow was in experiment 3. Here, due to a small incision made to expose the chorda, it was found difficult to adjust the current to the point where a copious flow of saliva was obtained without causing small muscular tremors at the same time. In view of this observation and because Bainbridge states in his original report that the variations in the rate of lymph flow were mainly dependent upon the strength of current used and only roughly correlated with the rate of salivary flow, stronger currents were used in the last two experiments. Muscular twitchings that occurred with the stronger current were roughly estimated by + for small tremors, + + + for rather violent contractions, and + + as midway between these two extremes.

As observed by Bainbridge, the increasing strength of current did increase the rate of lymph flow, but this increase occurred only with increasing muscular motion and appeared in no way related to salivary flow. The same results were obtained if the electrode was moved just off the chorda into the surrounding soft tissue (fig. 3).

Four experiments were then performed where pilocarpine hydrochloride in varying amounts (0.025 to 0.10 mgm. per kgm. of body weight) was given intravenously to stimulate salivary flow. It was found that even the smaller doses caused marked respiratory and vascular changes without any appreciable flow of saliva. Doses sufficient to cause a copious flow of saliva caused such respiratory embarrassment that the accessory muscles of respiration in the neck region became very active. The results were too confusing to permit discussion or tabulations.

SUMMARY AND CONCLUSIONS

In six experiments it has been impossible to confirm the reported increase in lymph flow from the cervical vessels after stimulating the submaxillary gland electrically except when the current used was sufficiently strong to cause simultaneous muscular activity.

Pilocarpine in amounts sufficient to cause a copious flow of saliva produced such marked respiratory and vascular changes that the data were unreliable as related to glandular activity and lymph flow.

These results are in entire agreement with the work of Carlson, Greer and Becht who in similar direct measurements failed to find that glandular activity increases lymph production and lymph flow.

The author is indebted to Dr. Cecil K. Drinker for suggesting this work and for his advice and assistance during this investigation.

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THE INFLUENCE OF HYPERPNEA AND OF VARIATIONS IN THE O₂ AND CO₂ TENSION IN THE INSPIRED AIR UPON NYSTAGMUS

ERNST GELLHORN AND IRWIN SPIESMAN

*The Departments of Physiology and Otolaryngology, College of Medicine, University of
Illinois, Chicago*

Received for publication May 10, 1935

In the two preceding papers of this series, the effects of the three factors mentioned in the title have been studied in regard to some visual and auditory processes, in order to investigate fundamental conditions of cortical excitability. The question now arises whether the results obtained in those experiments are applicable to the brain as a whole or are characteristic of the cortex. In order to decide this question experiments were undertaken in which the effects of O₂-lack, CO₂-excess and hyperpnea were studied on a typical brain stem reflex, caloric nystagmus. Magnus (1924) has shown that nystagmus is present after the removal of the cerebellum. Furthermore, according to Kubo (1906) and Bauer and Leidler (1912), even the removal of the tel- and diencephalon does not interfere with the occurrence of caloric nystagmus (for further details compare Spiegel, 1931).

METHOD. We used the method of Veits (1928) by which even weak stimuli cause nystagmus. Five cubic centimeters of water of a temperature varying between 28° and 32°C. were injected through an ear funnel toward the posterior upper wall of the external auditory meatus. The temperature and speed of the flow of the water were rigidly controlled so that the injection lasted exactly ten seconds. During the injection, and for 48 seconds afterward the experimental subject was seated on a Barany chair, leaning his forehead against a support which kept the head bent forward 30°. After 48 seconds the head was bent backward by the experimenter in 2 seconds, so that the head rested in a position which was 90° different from the preceding one. The experimental subject directed his eyes toward the left and kept them in this position while the experimenter counted the number of nystagmic movements through a Frenzel lens. The nystagmus was recorded by the experimenter with a key and signal magnet on a kymograph. The stimuli are so mild that the experimental subject has no other sensation than that of slight coolness in the ear during the injection. In no case was vertigo observed. If controls are carried

out at intervals of 30 minutes and more, they show very great consistency; in fact the records taken over a period of weeks seem to indicate that under well-controlled conditions the number of nystagmi is very constant for each individual. The latent period of the nystagmus was also studied but did not show any significant changes. This is probably due to the fact that the nystagmus occurred with a very short latency immediately after the subject has taken the correct position and moved the eyes to the left.

RESULTS. The experiments on the influence of O_2 -lack, CO_2 -excess and hyperpnea on nystagmus are based on 325 observations on 6 trained subjects. Ninety-six preliminary tests were made in order to establish the technique. Hereafter 25 experiments with hyperpnea, 18 with CO_2 -excess and 18 with O_2 -lack were performed, which, due to the fact that each experiment was preceded and followed by one to two controls, comprised a total of 229 tests.

In the first group of experiments the influence of CO_2 -excess was studied. Five per cent to 7 per cent CO_2 -air-mixtures were inhaled from a Douglas bag for 4 to 7 minutes. Immediately thereafter the caloric stimulation was performed. Figure 1 shows that the number of nystagmi is decreased immediately after CO_2 administration. Frequently there is no essential difference in the time during which nystagmi are observed, but the rate of nystagmic movements is considerably slowed. From the graphs of figure 1, it is apparent that the same person shows only slight differences in the controls, although the individual experiments were separated by several days and sometimes weeks.

The second group of experiments concerns the influence of hyperpnea. The experimental subject breathes maximally at a rate of 35 per minute for 2 or 3 minutes. Immediately thereafter the nystagmus was investigated. Also, in this case the effect was clear cut, showing a regular increase in the number of nystagmic movements in all experiments (fig. 2). The rate of nystagmi was not significantly altered.

In contrast to the two groups of experiments described above it may be stated that it was very difficult to get clear results with experiments on the effects of O_2 -lack on nystagmus. In the first group of six experiments, $9\frac{1}{2}$ to $10\frac{1}{2}$ per cent O_2 - N_2 -mixtures were inhaled from a Douglas bag from 7 to 12 minutes. In 4 of these experiments the nystagmus was unaltered; in 1 a slight increase was observed and in 1 a decrease. Obviously, the degree of anoxemia produced by the inhalation of O_2 in the concentrations mentioned does not lead to any impairment in the function of the brain stem, although it causes considerable changes in hearing and vision. We tried, therefore, to produce an anoxemia of a considerably longer duration or intensity. Nine experiments of this type were carried out on six persons (table 1). In these experiments the experimental sub-

ject inhaled O_2-N_2 -mixture of 8 per cent to 10 per cent from 2 to 3 Douglas bags, for as long as 50 minutes. In 6 experiments (on 5 experimental subjects) a decrease in the number of nystagmi was observed, which was

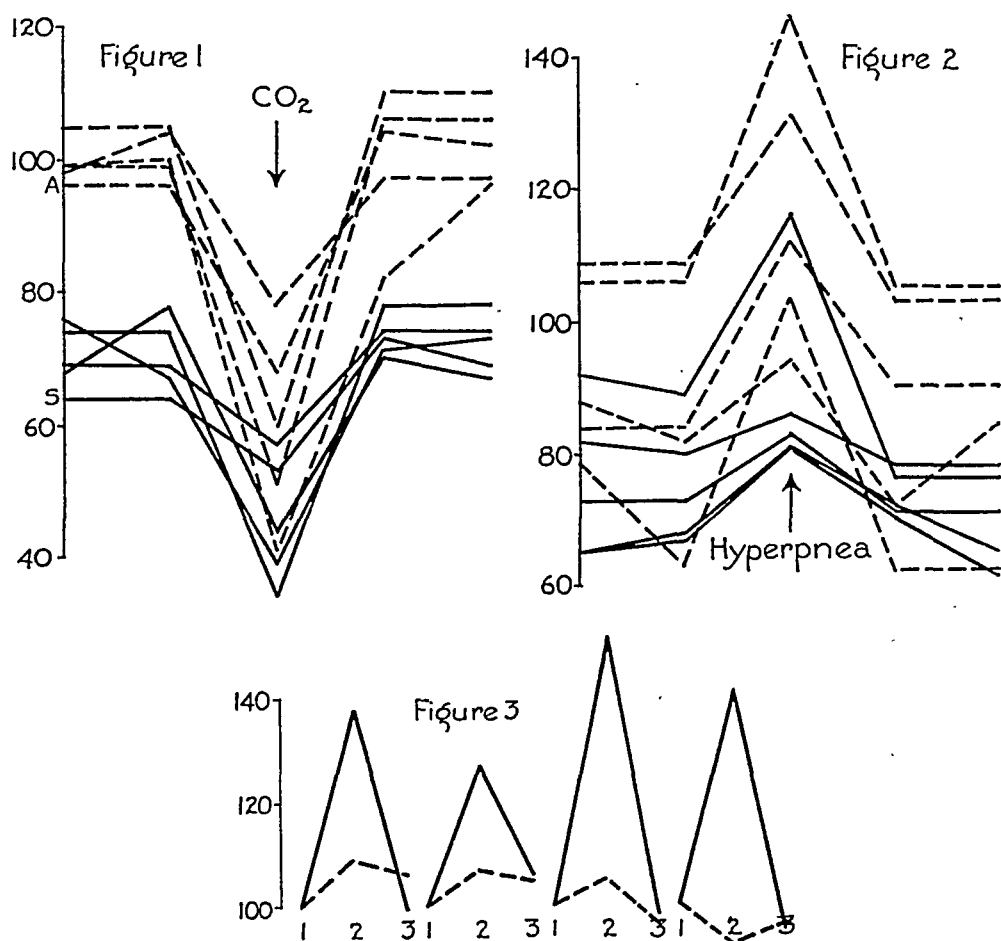


Fig. 1. The influence of CO_2 on the number of nystagmic movements after caloric stimulation. The CO_2 -experiment was preceded and followed by two control experiments in which the experimental subject breathed air. The interrupted lines represent experiments with A. J.; the solid lines those with L. S.

Fig. 2. The influence of hyperpnea on the number of nystagmic movements.

Fig. 3. The influence of time on the effect of hyperpnea in regard to nystagmus. Experiments with 4 subjects; 1 and 3 controls, 2 after hyperpnea. Solid lines: caloric stimulation carried out immediately after hyperpnea; interrupted lines: caloric stimulation carried out 3 minutes after the end of the hyperpnea period.

completely reversible when, after thirty minutes of atmospheric air, a control stimulation was performed (nos. 1-6 in table 1). But even in these successful experiments the effect (with the exception of no. 5) was very

slight, compared with the considerable changes obtained by CO_2 -excess and hyperpnea.

The experiments done with Mr. M. (nos. 7-9) are particularly interesting. This young man of an unusual physical fitness inhaled 10 per cent O_2 for as long as 50 minutes without experiencing any discomfort. However, in experiments on the latent period, duration, and intensity of after-images under the influence of O_2 -lack, he showed the typical effects described in the preceding papers after inhalation of O_2 for as short a time as 6 or 7 minutes. Moreover, he gave the typical results with CO_2 -excess and hyperpnea on nystagmus. In a final experiment he was subjected to an O_2 - N_2 -mixture of 8 per cent (no. 9). The experiment had to be discontinued after 6.2 minutes because of the severe symptoms. The face was extremely pale, the fingernails blue, he was nauseated and had the

TABLE 1
The influence of O_2 -lack on nystagmus

NUMBER	EXPERIMENTAL SUBJECT	NUMBER OF NYSTAGMI			O_2 -CONCENTRATION	DURATION OF O_2 -LACK
		1. Control before O_2 -lack	2. Immediately after O_2 -lack	3. Control after O_2 -lack		
					<i>per cent</i>	<i>minutes</i>
1	St.	92	72	95	10.1	19
2	J.	105	94	109	9.9	15
3	K.	114	91	103	10.0	25
4	Ma.	87	69	90	10.5	12.5
5	Ma.	88	39		10.1	25.5
6	Kan.	106	94	109	10.0	22.5
7	Me.	92	93	90	9.87	30.0
8	Me.	97	98		10.5	50.0
9	Me.	97	96	106	8.0	6.3

feeling of faintness. But the caloric stimulation performed immediately after the O_2 -lack period had been discontinued did not show any change at all.

Since in the previous papers it has been shown that the effects of hyperpnea and O_2 -lack lasted considerably longer than the duration of the experiment, the question was investigated whether such after-effects could be found in brain stem experiments. Since the O_2 -lack effect on nystagmus was too small and since the CO_2 -effect was temporary even in experiments with vision and hearing, we decided to study this question in regard to the effect of hyperpnea on nystagmus. The experiments were run in the usual manner, except for the fact that the caloric stimulation was not made immediately after the hyperpnea period was over, but three minutes later. All experiments had the same result. The number of nystagmi was the

same as in the controls, indicating that the effect of hyperpnea on nystagmus had completely ceased in this short interval (fig. 3).

DISCUSSION. The outstanding difference in the reaction of the brain stem investigated by the nystagmus reflex, and of the sensory cortex, studied in regard to auditory threshold and visual after-images in the preceding papers, is the fact that the effect of O_2 -lack is very profound on cortical phenomena and comparatively small on a function involving sub-cortical structures. This observation is in agreement with those of other authors (literature by Gildea and Cobb, 1930) according to which the cortex of the brain is most sensitive to O_2 -lack, whereas brain stem and spinal cord show a considerably less degree of sensitivity. Our experiments show that similar relations hold true for the human brain. If conditions are chosen with prolonged O_2 -lack, the nystagmus decreases. The phenomenon seems to indicate a decrease of excitability of the nervous mechanism involved, which is comparable but quantitatively different from similar changes in the excitability of the cortex.

The effect of CO_2 -excess resulting in a diminution of the number of nystagmic movements seems also to harmonize with the decreased excitability of the cortex under similar conditions. There is, however, a fundamental difference between cortex and brain stem in their reactivity to hyperpnea. Whereas hyperpnea leads to a decrease in cortical excitability, it increases the brain stem reflex (nystagmus) considerably. The brain stem reacts as does the spinal cord, since King, Garrey and Bryan (in animals) and Strughold and Jörg (in man) have found that hyperpnea increases tendon reflexes. The discrepancy of the results obtained with the cortex and with the brain stem seems to be explainable on the basis of the assumption of a differential sensitivity to O_2 -lack. As has been discussed in detail in the preceding papers, we know, on the basis of Cobb's and Schmidt's experiments, that hyperpnea leads to a vasoconstriction. The effect of hyperpnea on nervous tissue is the resultant of two antagonistic factors: First, of the specific increase in excitability of the nervous tissue whenever the CO_2 -tension in the blood is decreased in hyperpnea, and, second, of the vasoconstriction which diminishes the O_2 -supply to the tissues. If the sensitivity of the nervous tissue to the latter factor is very great, as is the case with the cortex, the vasoconstriction may outweigh the other factor, and as a result, we have a decrease in excitability. If, however, the O_2 -sensitivity is not great, as has been shown to be true for the brain stem, the specific effect of the decreased CO_2 -tension on the nervous tissue must result in an increased excitability.

Taking into account the great differences in sensitivity of various parts of the central nervous system to O_2 -lack, we come to an understanding even of their qualitatively different reactions on a uniform basis. It seems, however, not unimportant to stress the significant differences at various

levels of nervous activity. Here should be mentioned the fact that after-effects are very marked in regard to auditory and visual processes after O_2 -lack or hyperpnea, but cannot be detected in similar experiments on nystagmus. This difference must be due either to differences in the sense organs or in the central nervous mechanisms involved. Whereas it is very difficult and uncertain to separate in vision and hearing peripheral processes in the sense organs from those occurring in the cortex following the stimulation of the former, such an attempt may be successful in regard to the effects of vestibular stimulation. Mowrer (1935) has recently shown that the electric discharges of the vestibular nerve resulting from rotation cease completely immediately after the cessation of the stimulus (rotation). The nystagmus must, therefore, be due to an after-discharge of the central neurons. This interpretation agrees well with findings of Fischer and Oldberg (1932) who emphasize the importance of the central neurons for the intensity of the nystagmus reaction. In the light of these observations it is very probable that O_2 -lack, CO_2 -excess and hyperpnea chiefly affect the central neurons. The great difference in sensitivity to O_2 -lack in the various reactions studied allows us to specify the location of the important neurons or synapses still further: They are probably cortical in the cases of vision and hearing and subcortical in nystagmus. We come to the conclusion that besides the qualitatively different reaction between cortex and subcortex to hyperpnea, there are considerable quantitative differences not only in regard to the immediate effect of O_2 -lack but also in regard to its after-effects, which are more marked in the cortex. It is, moreover, not improbable that the differing effects of hyperpnea on the several processes are due in the last analysis to the different reactions of the respective neurons to O_2 -lack, which plays an indirect rôle in hyperpnea because of resultant vasoconstriction (compare the first paper of this series). The CO_2 -effect is found to be the same for the brain stem as it is for the cortex. Here we are concerned with the effect of the changes in CO_2 -tension on the excitability of nervous tissue. These changes, of course, lead to alterations in hydrogen ion concentration, but we have not attempted to differentiate effects of each, if indeed the CO_2 -tension has any effect excepting that through the changes in pH, which in turn affects such factors as the ionization of calcium.

SUMMARY

In studies on the effect of CO_2 -excess, O_2 -lack and hyperpnea on nystagmus in the human, it was found that breathing CO_2 -air-mixtures results in a decrease in the number of nystagmic movements and that hyperpnea produces an increase in this number. The brain stem reacts to increases and decreases in the CO_2 -tension of the blood with the same direction of change in excitability as occurs with spinal reflexes. O_2 -lack produces

an effect on the nystagmus (decrease in number) only under the conditions of severe anoxemia. The effects are temporary, and as soon as 3 minutes afterwards control values are obtained. These reactions are strikingly different from those observed in the case of the more strictly cortical processes reported in the preceding papers. The differences appear to be largely associated with the relative sensitivity to O_2 -lack. The cortical neurones are extremely sensitive to alterations in oxygen supply, while the brain stem centers studied, either because of a more adequate blood supply, or for other reasons, are relatively insensitive to such changes.

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THE INFLUENCE OF SODIUM TAUROCHOLATE, HEPATIC BILE AND GALL-BLADDER BILE UPON THE ABSORPTION OF OLEIC ACID FROM THE SMALL INTESTINE¹

CECILIA RIEGEL, K. O'SHEA ELSOM AND I. S. RAVDIN

From the Departments of Research Surgery and of Research Medicine, and from the Gastro-Intestinal Section of the Medical Clinic of the University Hospital, University of Pennsylvania

Received for publication May 10, 1935

This communication is a report of experiments made to determine the effect of sodium taurocholate, of hepatic bile and of gall-bladder bile upon the absorption of oleic acid from isolated jejunal loops prepared in dogs by the method of Johnston (1). Intestinal loops so made permit a more accurate quantitative determination of absorption from the small intestine than was possible in the few earlier reports dealing with this problem (Plant, 2; Verzar and Kuthy, 3). In the experiments here reported it was found that the absorption of oleic acid, negligible when this substance was introduced alone, was greatly increased in the presence of sodium taurocholate, slightly less so in the presence of hepatic or of gall-bladder bile.

Experiments were made upon 4 dogs. A constant quantity of oleic acid, 869 mgm., was introduced into the loops alone or with either sodium taurocholate, hepatic bile or gall-bladder bile. The amount of sodium taurocholate employed varied from 82 to 246 mgm. Normal dog's bile, hepatic or gall-bladder, was varied in amount so that the taurocholate content of the bile introduced was between 166 and 275 mgm. Tap water was used to bring to 30 cc. the total volume of fluid introduced in each experiment. At the beginning of each experiment, therefore, the oleic acid concentration was 0.102 mole per liter, while the taurocholate varied from 0.005 to 0.016 mole per liter.

Oleic acid was introduced into and removed immediately from the loops in a series of control observations upon each dog. In all other experiments the loops were closed for 1, 2 or 3 hours after which they were emptied, thoroughly washed with water and the washings added to the material originally recovered. This material was quantitatively analyzed for its oleic acid content. The fatty acid was saponified with potassium hydroxide, acidified with hydrochloric acid and extracted with petroleum ether. Aliquot portions of the petroleum ether extract were evaporated to dryness,

¹ Aided by a grant from the Faculty Research Committee.

taken up in 95 per cent alcohol and titrated with standard sodium hydroxide. This method permits recovery of 95 per cent of a known quantity of oleic acid added to intestinal juice *in vitro*. Intestinal juice alone thus tested contained a small amount of material reacting as fatty acid. Since the amount present was relatively constant and did not exceed 3 per cent of the total oleic acid used in the experiments it has been disregarded. The material withdrawn after introduction of bile salt or bile

TABLE 1

Per cent of oleic acid not recovered from intestinal loops when introduced alone or in combination with sodium taurocholate, hepatic bile or gall-bladder bile

MATERIALS USED	MEAN PER CENT LOSS				
	Dog 1	Dog 2	Dog 3	Dog 4	Average all experiments
Removed immediately					
Oleic acid (869 mgm. used in all experiments)	5.9 \pm 0.8* (6)†	12.4 \pm 2.1 (4)	11.2 \pm 2.6 (10)	6.6 \pm 1.5 (6)	9.1 \pm 1.2 (26)
Removed after 2 hours					
Oleic acid	15.4 \pm 1.4 (12)	10.6 \pm 2.0 (7)	12.2 \pm 6.5 (4)	10.9 \pm 1.9 (6)	12.9 \pm 3.7 (29)
Oleic acid + sodium taurocholate (82-246 mgm.)	45.6 \pm 2.0 (21)	36.7 \pm 3.6 (5)	21.8 \pm 1.2 (7)	28.4 \pm 3.5 (4)	38.0 \pm 2.1 (37)
Oleic acid + hepatic bile (taurocholate content: 166-275 mgm.)	41.9 \pm 4.1 (5)	19.0 (1)	13.0 \pm 3.5 (5)		26.7 \pm 5.0 (11)
Oleic acid + gall-bladder bile (taurocholate content 166-275 mgm.)	35.7 \pm 2.2 (7)	23.0 \pm 3.9 (6)	16.6 \pm 2.3 (6)		25.7 \pm 2.4 (19)

$$* \text{ S.E. of mean } = \sqrt{\frac{\sum d^2}{n(n-1)}}$$

† = Number of experiments entering into the mean.

was analysed also for taurocholate but the method of analysis on mixtures containing intestinal juice was subject to large errors and, therefore, quantitative figures are not given.

From the results presented in table 1 it will be seen that:

1. The dogs often differ significantly from one another in the degree of their response to a given procedure. The averaging together of the experiments on different dogs is, therefore, open to criticism.

2. Comparison of the data obtained when oleic acid was removed imme-

diately with those secured after oleic acid alone had been in the loop 2 hours, shows that in dogs 2 and 3 the percentage of oleic acid lost is not significantly different in the two periods and there is, therefore, no definite evidence of absorption when oleic acid alone was used. In dogs 1 and 4 slight absorption of oleic acid when introduced alone cannot be denied but the evidence that it occurred is not convincing.

TABLE 2

Absorption of oleic acid (869 mgm. introduced) under different conditions, and after varying intervals in dog 1

OLEIC ACID		OLEIC ACID + HEPATIC BILE		OLEIC ACID + GALL BLADDER BILE	
Immediate withdrawal	2 hours (12 observations)	Removed after 2 hours		Removed after 2 hours	
Lost	Lost	Sodium taurocholate introduced	Oleic acid lost	Sodium taurocholate introduced	Oleic acid lost
<i>per cent</i>	<i>per cent</i>	<i>mgm.</i>	<i>per cent</i>	<i>mgm.</i>	<i>per cent</i>
6.3	9.0 to 26.4	165	53	166	35
9.3		165	43	166	30
4.6	Av. = 15.5	255	30	174	36
5.5		255	37	210	48
4.0	S.D. 4.7†			275	33
5.5					

OLEIC ACID + SODIUM TAUROCHOLATE

Removed after 1 hour		Removed after 2 hours			Removed after 3 hours	
Sodium taurocholate introduced	Oleic acid lost	Number of experiments	Sodium taurocholate introduced	Oleic acid lost	Sodium taurocholate introduced	Oleic acid lost
<i>mgm.</i>	<i>per cent</i>		<i>mgm.</i>	<i>per cent</i>	<i>mgm.</i>	<i>per cent</i>
165	25	6	82	52 ± 3*	165	36
165	24	4	100-110	44 ± 3	165	39
165	28	4	166-186	40 ± 4	165	50
		3	246	40 ± 6		

$$* \text{ S.E. of mean} = \sqrt{\frac{\sum d^2}{n(n-1)}}$$

$$\dagger \text{ S.D. of observations} = \sqrt{\frac{\sum d^2}{n-1}}$$

3. Comparing the influence of hepatic and gall-bladder bile on absorption of oleic acid, in none of the dogs is there a significant difference in the effect of the two types of bile when the total bile salt content is about the same. In dog 3 absorption is not significantly demonstrated in either instance; in dog 2 absorption is definite though small and in dog 1 it is marked.

4. In the experiments with sodium taurocholate all the dogs exhibit the

same order of increased absorption, this being definitely greater than with bile although in dog 1 the difference is hardly significant.

From table 2 it is evident that:

1. There is no correlation between the amount of sodium taurocholate in excess of 82 mgm. introduced and the per cent of oleic acid absorbed.

2. The absorption of oleic acid continues for at least two hours but the absorption in 3 hours is not demonstrably greater than at the end of 2 hours.

The relative concentrations of oleic acid and taurocholate in the fluid introduced into the loops preclude any direct chemical reaction between the two as an explanation for the absorption of oleic acid. During the periods of closure of the loop, fluid was poured into the loop in varying quantities. At the end of 1 hour the average quantity of fluid withdrawn was 62 cc., the concentration of oleic acid 0.038 mole per liter and approximately half of the bile salt had been absorbed. The fact that absorption proceeded for an additional hour indicates that even a low concentration of taurocholate was effective during the second hour. At the end of 3 hours taurocholate if present was found in only very small amounts and no evidence was obtained that fat was absorbed during this period. No correlation was found between the amount of fluid withdrawn from the loop either with or without taurocholate and the total quantity of oleic acid absorbed. It is apparent, therefore, that the action of taurocholate in facilitating the absorption of oleic acid must be in the nature of a surface phenomenon and that very minute concentrations are sufficient to produce absorption of relatively large quantities of fatty acid.

CONCLUSIONS

From the foregoing experiments it is concluded that the absorption of oleic acid is negligible when introduced alone into a loop of small intestine, is greatly increased by the presence in the loop of sodium taurocholate, and somewhat less so by hepatic bile or gall-bladder bile.

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FURTHER STUDIES OF THE EFFECTS OF THE ESTROGENIC AND THE GALACTOPOIETIC HORMONES UPON THE MAMMARY GLAND OF THE RABBIT

W. U. GARDNER,¹ E. T. GOMEZ AND C. W. TURNER

From the Department of Anatomy, Yale University, School of Medicine, and from the Dairy Husbandry Department, Agricultural Experiment Station, University of Missouri, Journal Series Paper No. 402

Received for publication May 3, 1935

The influence of the estrogenic hormone (ovarian follicular hormone or theelin) upon the stimulation of mammary growth has been extensively investigated in several species of laboratory animals (Turner et al., 1930-1934). The normal development of the mammary glands of these species have also been studied (Turner et al. as cited above). Theelin induced a growth of the mammary duct system but did not induce an appreciable development of the alveoli, except in the guinea pig. The type of development of the mammary glands induced by theelin was comparable to the normal development observed in post-pubertal virgin animals.

The hormone of the corpus luteum was necessary to produce the further development of the mammary glands (Turner et al., 1931-1934). The experimental development of the glands may thus be divided into a stage of duct development induced by theelin and a stage of alveolar development requiring the hormone of the corpus luteum in addition to theelin. In the normally developing mammary glands the sudden development of the alveolar elements occurred during pseudo-pregnancy or during the first half of pregnancy (fig. 4).

Lactation was not observed following the administration of the ovarian hormones. Stricker and Grueter (1928) first demonstrated that a preparation of the anterior pituitary induced lactation. This observation has been verified and extended (Corner, 1930; Asdell, 1931; Turner and Gardner, 1931; Nelson and Piffner, 1931; Catchpole and Lyons, 1933; Gardner and Turner, 1933; Riddle, Bates and Dykshorn, 1933; Lyons and Catchpole, 1933; Donahue, 1934).

Corner (1930) was of the opinion that the pituitary would also induce growth of the mammary glands. Asdell (1931) observed that immature female rabbits did not respond to a similar pituitary preparation. Lyons and Catchpole (1933) reported obtaining lactation and mammary growth

¹ National Research Council Fellow.

in mature castrate virgin rabbits. In contrast to the above observers, Gardner and Turner (1933) concluded that the pituitary did not stimulate a real growth of the mammary glands but acted on the existing epithelium stimulating secretory activity. The involuted glands of old castrate or the glands of ovariectomized immature rabbits were not developed or were not stimulated to secretory activity by galactin except in one rabbit when theelin preceded galactin treatment. The enlargement of the mammary glands following such treatment was the result of distention with secretion rather than a true growth stimulation. The marked thickening of the mammary glands observed during normal lactation was never observed in such animals without the previous existence of the alveoli.

The present investigation was undertaken to further study the specificity of the anterior pituitary galactopoietic hormone and the effect of this hormone on the mammary gland duct system.

MATERIALS AND TECHNIQUE. Immature and mature castrate virgin female, castrate multiparous, and normal and castrate male rabbits were used in the following experiments. Thus the effect of the galactopoietic hormone was investigated upon the partially developed mammary glands of young and mature male and female rabbits.

A total of 47 experiments were performed using 43 rabbits. The duration and rate of theelin² injections varied in different experiments as will be indicated later. The amounts of lactation stimulating hormone used were tested and known to contain in excess of one rabbit unit.

The developmental changes of the glands induced by theelin injections were determined either on the removed glands or by observations at laparotomy. The removed glands were studied either as whole gland preparations or were sectioned for histological examination, or both. The degree of response to the lactation stimulating hormone was determined by observation. Histological preparations of such glands were used to further portray their structure.

Galactin treatment following the ovariectomy of young virgin rabbits. Four rabbits were used in this series. Though estrus was not tested by attempted mating in two animals it is probable they were all in estrus at the time they were ovariectomized. The mammary glands removed at the time of ovariectomy consisted of an extensive duct system (figs. 1 to 3). A typical gland removed from one of the rabbits at this time measured 3×5 cm.

Three rabbits received galactin during the 6 or 7 days immediately

² The estrogenic hormone used was theelin. This was kindly supplied by Parke Davis Co. The galactopoietic hormone (galactin) was largely prepared by modifications of methods previously described by the writers. Four male rabbits received "Prolactin." This extract was kindly supplied by Dr. J. F. Anderson, of E. R. Squibb and Sons.

following ovariectomy. The mammary glands of one injected and of the uninjected rabbit were examined on the third day and found to contain a serous secretion. On the day following the cessation of injections the mammary glands of the injected rabbits were all distended with a milk-like fluid. The main ducts were enlarged and together with the dilated small lateral ducts and sprouts gave the gross impression of a fully lactating gland. Though some cellular hyperplasia may have occurred it had not been extensive enough to change the type of mammary structure which was still essentially a compound tubular gland. The mammary glands of the uninjected control rabbit still contained some clear fluid on the seventh day.

This experiment confirms in part the observations of Corner (1930), Asdell (1931) and Catchpole and Lyons (1933). The effect of the lactation stimulating hormone, however, was assigned to a mammary hypertrophy resulting from the accumulation of secretion rather than to both a growth and a secretory stimulation.

The effect of galactin following theelin injections on the mammary glands of immature ovariectomized rabbits. It has been previously demonstrated (Asdell, 1931; Gardner and Turner, 1933) that the mammary glands of immature female rabbits do not respond to galactin. The mammary glands of such rabbits consisted of duct systems extending less than 1 or 2 cm. beyond the base of the nipple. Though the ovaries of such rabbits may possess follicles their hormone production must be quite limited as the mammary glands develop slowly during the pre-pubertal period. If the growth of the mammary glands were suddenly accelerated would they respond to galactin?

Fourteen immature female rabbits were used. Twelve of the animals were from 3 to 4 months old while two were somewhat older. The ovaries of the twelve younger rabbits contained no large follicles at the time of ovariectomy. Several moderately large follicles were observed in the ovaries of the two older rabbits. The control mammary glands removed from the younger rabbits extended but slightly beyond the base of the nipple. Twenty and 25 rat units of theelin were given daily for 20 days starting immediately after ovariectomy. Examination of the mammary glands following theelin injections showed that a marked growth had been induced. The glands measured from 2 to 6 cm. in diameter. In a few cases some serous fluid was present in the ducts.

An excess of one rabbit unit of galactin was given during 6 and 7 days for the younger and older rabbits, respectively. The mammary glands were again examined the day following the cessation of galactin treatment. Again there was no evidence of growth of the mammary glands following the discontinuance of the theelin injections. In all instances the ducts were distended with milk or a milk-like secretion. The results were es-

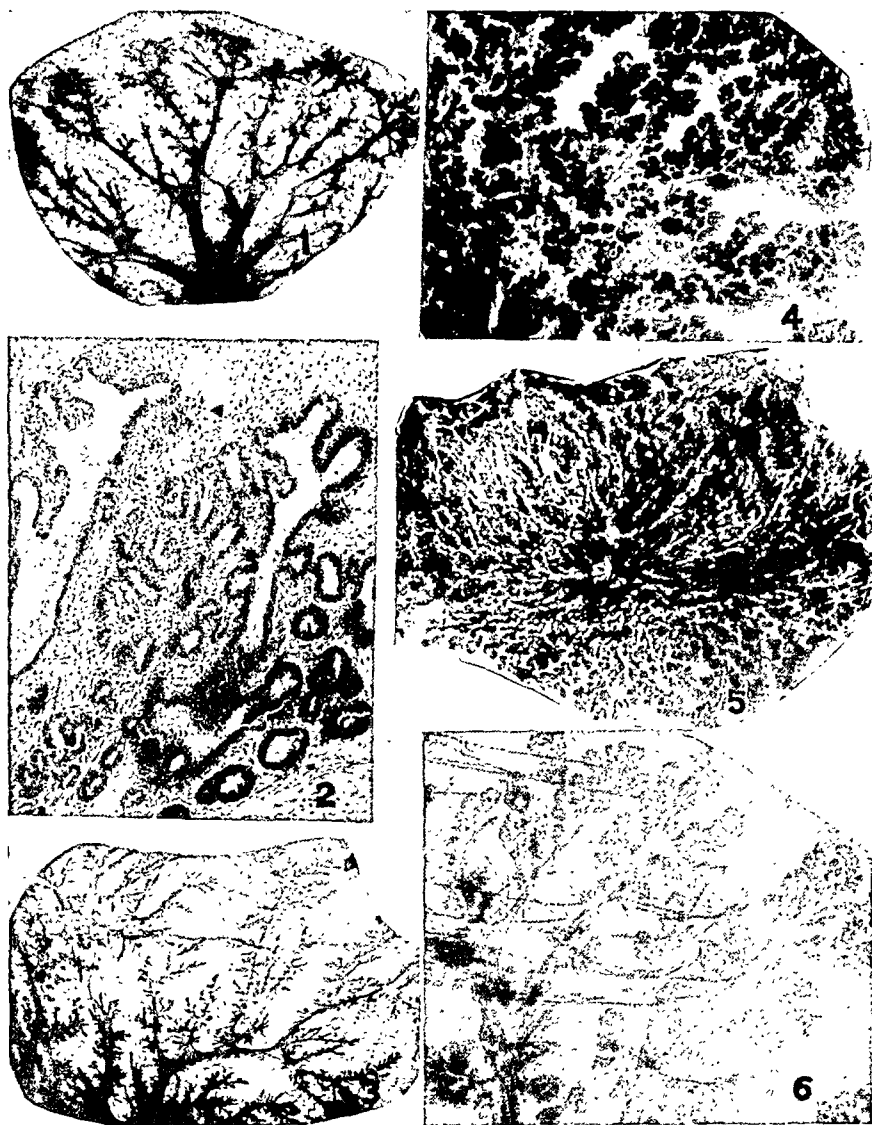


Plate I. Fig. 1. Microphotograph of a portion of the mammary gland of a sexually mature nulliparous rabbit. The gland was removed immediately after copulation. $\times 4$.

Fig. 2. Microphotograph of portion of the sectioned mammary gland shown in figure 1. $\times 6.5$.

Fig. 3. Microphotograph of a portion of the mammary gland of an adult nulliparous rabbit during anestrus. $\times 2.6$.

Fig. 4. Microphotograph of a portion of the mammary gland of a rabbit at the end of pseudo-pregnancy (15 days). $\times 2.6$.

Fig. 5. Microphotograph of a whole mount of the mammary gland of the rabbit at the time of parturition. The milk was expressed from the gland before fixation and staining. See figure 7 for section. $\times .65$.

Fig. 6. Microphotograph of a portion of the mammary gland of a sexually mature

nulliparous rabbit which received 20 rat units of theelin daily for 20 days followed by 2 cc. of galactin administered once daily for six days. Note the secretion in the lumina of the large ducts and slight hypertrophy of the lateral and peripheral outgrowths. See figure 9 for section. $\times 2.6$.

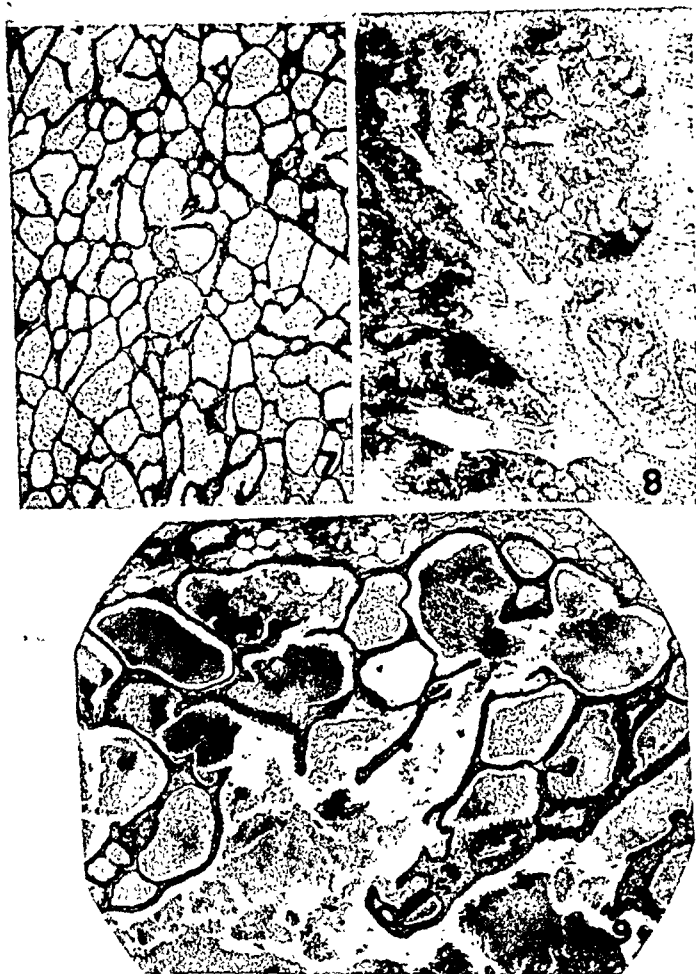


Plate II. Fig. 7. Section of lactating rabbit mammary gland. Lactation was induced by galactin at the end of pseudo-pregnancy. $\times 19.5$.

Fig. 8. Section of the mammary gland of a young male rabbit which received 20 rat units of theelin for 20 days followed by 2 cc. of galactin daily for six days. Note the secretion in the lumina of the ducts and the multilayered epithelium of the walls of the lateral branches. $\times 19.5$.

Fig. 9. Section of the lactating mammary gland of the sexually mature female rabbit shown in figure 6. Note dilatation of the duct end buds in comparison with the alveoli of a fully grown (lobular-alveolar system) and lactating mammary gland. $\times 19.5$.

entially similar to the previous series except that the amount of distention of the glands was not as marked as in the proceeding instance. Whereas the extent of lactation was rated as plus two (+ +) in the previous experiment it was rated as plus one (+) in the present series.

The effect of galactin following theelin upon the regressed mammary glands of ovariectomized multiparous rabbits. Following ovariectomy the mammary glands of multiparous female rabbits undergo an extensive involution. Within a few months only the duct system remains and this also slowly regresses so that finally only the larger mammary ducts persist. From the viewpoint of mammary gland structure, the regressed mammary glands of ovariectomized multiparous rabbits are comparable with those of mature virgin females (Turner, 1932; Gardner and Turner, 1933).

Eleven multiparous rabbits whose mammary glands had been allowed to regress for at least 100 days were used in the present series of experiments. Four of these rabbits were given daily injections of 20 rat units of an oil solution of theelin for 10 days followed by one rabbit unit of galactin during six days. Secretion was not observed in the mammary gland ducts following the theelin injections and in the mammary glands of only one rabbit following the galactin injections. The amount of secretion in this case was very slight.

A second series of six similar rabbits was similarly treated except the theelin injections were continued for 20 days and two of the rabbits received 25 rat units of water soluble hormone daily. The mammary ducts of the two rabbits receiving the 25 rat units of theelin contained a small amount of a serous secretion the day the injections were stopped. Following the injection of one rabbit unit of galactin daily during six days the mammary ducts of all the rabbits were distended with the contained milk (figs. 6 and 9).

One similar rabbit received galactin without previous theelin treatment. Secretion was not observed in this animal.

As in the two preceding series of experiments galactin was observed to be effective in inducing lactation after the duct system had been under the influence of theelin. Also, as in the previous series, there was no evidence that the galactopoietic factor induced the growth of the glands. The changes in the glands were the result of the distention of the mammary ducts. The ten day period of theelin injections was concluded to be too brief to sufficiently repair or condition the glands to respond to galactin.

The effect of galactin following theelin on the mammary glands of normal and castrate male rabbits. The rudimentary mammary glands of nine male rabbits, five of which were normal and four of which were castrated, were developed by theelin stimulation. The experiments were repeated in two of the normal males a second time so a total of 11 experiments were performed.

One normal and one castrate male were treated with 20 rat units of theelin for ten days. The mammary glands at this time were slightly developed. Galactin was then injected for six days. The mammary glands showed no evidence of further growth after the cessation of theelin injection. Again the ten day periods of injections were not sufficiently long to develop or condition the glands for any secretory response to galactin.

Seven of the rabbits received 20 or 25 rat units of theelin daily for 20 days. Two of the latter rabbits were later given 50 rat units of theelin daily for 14 days. The mammary duct system developed under the influence of theelin extended from 2 to 3 cm. out from the base of the nipple. In all cases the ducts were quite large and in some instances they were observed to contain a serous fluid.

Galactin (prolactin was given to four of the rabbits in this series) was given the rabbits immediately following the cessation of theelin treatment. After treatment for six to seven days definite secretory activity was observed in all cases. In some of the rabbits this consisted of the presence of milk or a milk-like fluid in the gland ducts (fig. 8). In other rabbits the ducts were greatly distended with milk.

The further growth of the mammary glands during the period of galactin or prolactin treatment was not observed. Likewise the extent of lactation observed never equaled that observed following the injection of galactin into male rabbits whose mammary lobules and alveoli had been developed in addition to the mammary ducts, by the simultaneous injections of theelin and progestin or corporin.

The effect of delaying galactin treatment following theelin. Is it necessary to start the injection of the galactopoietic hormone immediately following the cessation of theelin or will the mammary glands remain sensitive to galactin for a period of time?

Five multiparous female rabbits whose mammary glands were involuted for over 100 days were injected for 20 days with 20 rat units of theelin daily. One rabbit received galactin for six days beginning on the second day after theelin treatment was stopped, one on the third, and one on the fourth, etc. Lactation was induced in the rabbit in which galactin treatment was started on the second day following theelin injections. The mammary glands of the rabbit given galactin on the third day following the theelin treatment showed some indications of secretory activity following the galactin treatment. None of the other rabbits gave any indications of response.

Though this series of animals is very small it is indicated that the mammary glands rapidly regress to a point where they will not respond to galactin following the cessation of theelin treatment.

Re-initiation of lactation by galactin following theelin. It has been ob-

served that galactin would not induce a second lactation response in castrate female rabbits following an experimentally stimulated lactation. Normal female rabbits also failed to respond a second time until the ovaries had again been allowed to act upon the mammary glands (Gardner and Turner, 1933). The possibility that theelin might be effective in the development or conditioning of the glands to galactin following a previous experimentally induced lactation was investigated in two mature, ovariectomized, multiparous rabbits. Ten days after the onset of previous experimentally induced lactation 20 rat units of theelin were injected daily for 20 days. At the time theelin injections stopped the glands consisted of a well developed duct system. Some lobules were observed near the nipples. After injecting galactin for six days the entire mammary structure was again engorged with milk.

DISCUSSION. The normal development and function of the mammary glands has been experimentally reproduced in immaturesly ovariectomized or male rabbits (Turner and Frank, 1932; Gardner and Turner, 1933); guinea pigs (Nelson and Piffner, 1931; Nelson and Smelser, 1933; Turner and Gomez, 1934); and cats (Turner and DeMoss, 1934). Thus far the guinea pig is the only species extensively investigated in which the hormone of the corpus luteum has not been required in the stimulation of complete mammary gland growth. Theelin administration for as long as 120 days has not been observed to produce a development of the mammary glands of rabbits beyond the duct stage, whereas theelin and corporin injected simultaneously produced complete mammary growth (Turner and Frank, 1932). A similar limitation of theelin stimulation during long time experiments has also been observed in mice (Turner and Gomez, 1934; Gardner et al., 1934). Corporin alone was not effective in the stimulation of the mammary growth in the rabbit (Corner, 1930; Turner and Frank, 1931) but when given simultaneously with the theelin induced a complete mammary development (Turner and Frank, 1931, 1932). The primary ovarian follicular hormone and corporin (progesterin) thus induced the stimulation of complete mammary growth. Mammary growth is essentially under ovarian control as indicated by Ancel and Bouin (1911). There appears to be no experimental evidence for the statement of Evans (1935) concerning the "non-necessity of progesterin in mammary physiology." The specificity of response of the mammary glands to the ovarian follicular³ and luteal hormones is as definite as is the specificity of response of the uterus to the same hormones, particularly in the rabbit.

³ The writers are aware that "Theelin," the purified commercial hormone used may not be the same chemical substance found in the ovary. At this time the similarity is based on biological rather than chemical data. The biological similarity, however, is considered to justify the reference to theelin as an ovarian follicular hormone.

Asdell and Seidenstein (1935) observed that the mammary glands of hypophysectomized rabbits could be developed by the stimulus of the ovarian hormones, theelin and progestin. Jeffers (1935) also observed the further development of the mammary glands of rats hypophysectomized during pregnancy. The growth and initial secretory processes of the mammary glands of such rats progressed normally. The above observations indicate that the pituitary is not essential for normal mammary gland growth except indirectly through the ovary.

No evidence of mammary gland growth was observed following the cessation of theelin injections in the above experiments. Though the mammary glands were denser and sometimes were actually of somewhat greater extent it appeared that this was due essentially to a distention of the duct system with secretion. Examination of the glands removed before and after galactin injections fail to show an increase in number of the smaller ducts (figs. 1-6). Likewise, it is only rarely that a lobular structure is found following galactin injections when none were observed in the control tissue previously examined. Though there may be some cellular hyperplasia it is certainly of secondary importance and is not sufficient to change the glands from the theelin induced compound tubular structures (figs. 8-9). The mammary gland duct epithelium undoubtedly possesses secretory capacities. Weatherford (1929) noted histological evidence of secretory activity of the smaller ducts in normally lactating rats; that is, in animals with fully developed mammary glands. This might be expected as the mammary alveoli develop as small out-growths from the smaller ducts. Histologically the epithelium of the smaller ducts and alveoli are identical. The smaller ducts apparently function both in supplying more secretory epithelium and in conducting the secretion from the terminal structures.

In the normal animal the proper physiological condition for the activation of the secretory epithelium of the mammary glands is not reached until the complete or almost complete development of the mammary glands has been induced. Mammary secretory activity is not observed normally except following pregnancy and, in some species, as in the dog, and ferret, following pseudo-pregnancy (figs. 5 and 7). During pregnancy the physiological conditions are such that mammary gland growth is produced.

The observations of Corner (1930) and Asdell (1931) showed that lactation resulted in mature ovariectomized virgin rabbits following the administration of lactation stimulating hormone. Such rabbits, though they have extensively developed mammary duct systems, possess very few if any mammary alveoli. Since estrus is more or less continuous in the isolated mature virgin rabbit it is probable that the mammary glands were influenced optimally by the animal's own ovarian secretions up to

the time of ovariectomy. When such rabbits were treated with the lactation stimulating hormone immediately following ovariectomy it was probable that the glands were subjected to the influence of lactation stimulating hormone immediately following theelin. In other words, under certain conditions the existing epithelium of the partially developed mammary gland may be stimulated to functional activity.

The observations reported in the present paper verify the above conclusions. The mammary gland ducts of male rabbits grown during theelin treatment responded to the lactation stimulating hormone. Similar results were observed when galactin was given old rabbits whose duct systems had been partially restored following extensive castrate atrophy. The mammary ducts of ovariectomized immature rabbits also responded to galactin after their slow development had been accelerated by theelin.

The influence of theelin also appears to be required for a certain definite period before the mammary epithelium will respond to galactin. None of the rabbits responded when theelin had been injected for a period of only 10 days. The glands also appeared to become refractory to galactin very soon after theelin injections were stopped. The small series of experiments reported above gave completely negative results following the lapse of more than three days between the cessation of theelin treatment and the beginning of galactin treatment.

What effect theelin has upon the mammary duct epithelium in conditioning its response to the galactopoietic substance is not known. A serous fluid has been observed quite frequently in the ducts following theelin injections alone but this fluid is definitely distinct from colostrum or milk and is present only in relatively small quantities. Only after the administration of galactin has the presence of a substance grossly and microscopically resembling milk been observed.

As theelin does in some way prepare the partially developed mammary glands for response to galactin it might also be assumed to similarly prepare the fully developed glands.

SUMMARY

The mammary duct systems of ovariectomized immature rabbits and of male rabbits developed under the influence of theelin respond to the galactopoietic hormone of the pituitary. Likewise, the mammary duct systems persisting after previous ovariectomy of multiparous females respond to galactin after theelin stimulation.

The galactopoietic hormone produced no definite growth of the mammary glands. The mammary glands of all the rabbits consisting of duct systems prior to galactin injections consisted essentially of a duct system following the injections. The increased density and area of the glands

were attributed primarily to a distention of the preëxisting ducts with fluid.

A period of theelin treatment in excess of ten days was required before the mammary glands of rabbits, not in estrus or recently ovariectomized during estrus, would respond to galactin. Indications of secretory activation of the mammary glands were observed in all rabbits treated with galactin immediately following a previous 20 day period of theelin treatment.

It is indicated that the mammary glands of the theelin injected rabbits fail to secrete when galactin treatment is delayed for more than three days after the cessation of theelin injections.

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A STUDY OF THE COMPARATIVE PHYSIOLOGY OF THE GLOSSOPHARYNGEAL NERVE—RESPIRATORY REFLEX IN THE RABBIT, CAT AND DOG

HARRY A. TEITELBAUM¹ AND F. A. RIES

From the Department of Physiology, School of Medicine, University of Maryland

Received for publication April 24, 1935

During an investigation of "A pharyngeal inspiratory reflex of the cat" (11), our interest was aroused by an apparent variation in the reflex respiratory responses of certain laboratory animals. In the text-books compiled by Luciani (5), Stewart (10), Greene (2), Macleod (6), Howell (3), and Evans and Hartridge (1), it is stated that respiration is reflexly inhibited by stimulation of the central end of the glossopharyngeal nerve. Of those who investigated the rôle of the glossopharyngeal nerve in respiration, Schiff (9) noted that it causes a prolongation of inspiration in the rabbit; Knoll (4) produced marked inspiratory changes; and Markwald (7) obtained complete inhibition of respiration in the same animal. The authors (11) have been able to confirm Markwald's observations in the rabbit.

MATERIAL AND METHOD. Fourteen cats, 10 dogs and 6 rabbits were used in the experiments to be reported. Barbitol-Na was administered intravenously under ether, the dose being 275 mgm. per kilo. The ether was then discontinued. Faradic current of varying intensities was employed. Respiration was recorded by means of a tambour connected to a tracheal cannula.

RESULTS. *Rabbit.* Stimulation of the central end of the glossopharyngeal nerve causes inhibition of respiration in this animal, as is evident in a typical record illustrated in figure 1. The carotid sinus branch of the glossopharyngeal nerve may, however, cause an acceleration of respiration, as in figure 2. The response recorded in figure 1 confirms the earlier observations of Markwald (7). The inhibition, which may occur in either inspiration or expiration, has a duration of 2 to 3 respirations; and then respiration breaks through regardless of the prolongation of the stimulus.

Cat. Of the 14 cats, only 1 responded to stimulation of the central end of the glossopharyngeal nerve in a manner comparable to that obtained in the rabbit (fig. 3). In two other cats some inhibition was elicited, but

¹ Hitchcock Fellow in Gross Anatomy.

the response was not comparable to that of the rabbit; for it was interrupted by respiratory acceleration, as may be the case in the dog.

The reflex respiratory response to glossopharyngeal nerve stimulation in the cat is usually initiated by a deep inspiration, as depicted in figure 4. This inspiration may be followed by a more or less prolonged expiration which may be interpreted as a phase of incomplete inhibition. Following the prolonged expiration which may be interrupted by shallow, rapid gasps, there may be a short inhibitory phase which is followed by marked acceleration of respiration; or more frequently there is an immediate marked respiratory augmentation as in figure 4.

The accelerator response in the cat is primarily inspiratory in nature in most instances. While the rare inhibitory response, as in the case of the

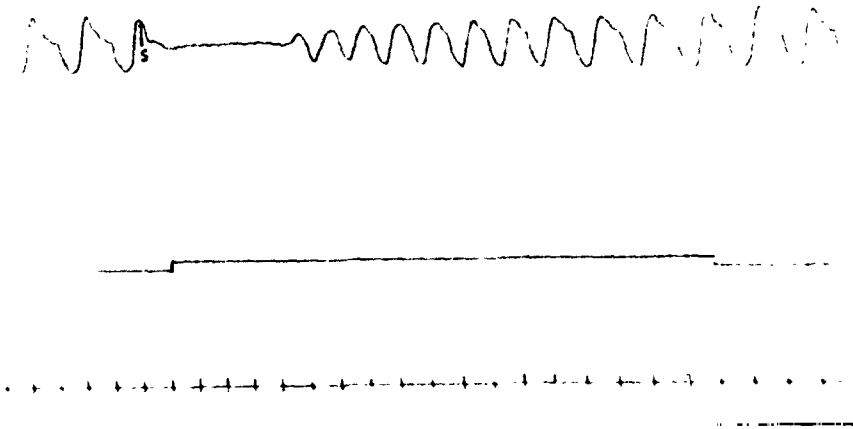


Fig. 1. Rabbit. Stimulation of the central end of the right glossopharyngeal nerve. Primary, 5 volts, secondary coil at 9 cm. Time, 1 second.

rabbit, has a short duration despite the prolongation of the stimulus, the more common accelerator response lasts as long as the stimulus is continued; being interrupted however by the element of fatigue if continued long enough. From the above it is quite evident that the anoxemia resulting from the respiratory inhibition is capable of overcoming the reflex nervous apnea despite the continuation of the electrical stimulus.

Dog. The respiratory response to central glossopharyngeal nerve stimulation is less constant in the dog than it is in the cat and rabbit. Of the 10 dogs studied, 7 responded with respiratory acceleration. Two of these gave a marked response, as illustrated in figure 5, on stimulation of both the right and left glossopharyngeal nerves. In a third dog the left nerve caused moderate acceleration, while the right caused slight inhibition of respiration. The 4 other animals, in addition to the accelerator response,

also evinced an inhibitory mechanism which involved either different branches of the same nerve, or different phases of the response to the stimulation of an individual nerve, as in figure 6.

Of the 10 dogs, 8 responded to central glossopharyngeal nerve stimulation with varying degrees of inhibition. The latter, as illustrated in

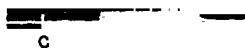
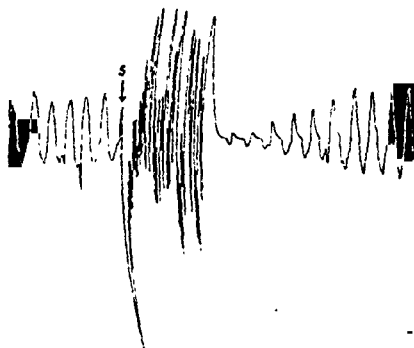
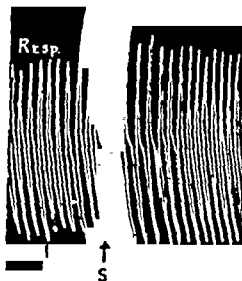
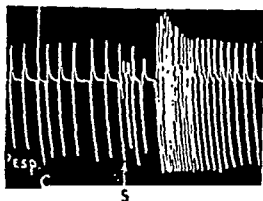


Fig. 2

Fig. 3

Fig. 4

Fig. 2. Rabbit. Stimulation of the central end of the carotid sinus branch of the right glossopharyngeal nerve. Primary, 5 volts, secondary coil at 11 cm. Time, 1 second.

Fig. 3. Cat. Stimulation of the central end of the right glossopharyngeal nerve. Primary, 6 volts, secondary coil at 10 cm. Time, 1 second.

Fig. 4. Cat. Stimulation of the central end of the right glossopharyngeal nerve. Primary, 6 volts, secondary coil at 12 cm. Time, 3 seconds.

figure 7, resembled that occurring in the rabbit in some cases. In others it was less marked. In 4 of the animals an accelerator response was also present, as in figure 6. In dogs in which the inhibitory response is not evident, as in the case illustrated in figure 5, it cannot be claimed that the mechanism involved is altogether absent; for the nature of the distribution

of the fibers might be such, that the accelerator response conceals the one of inhibition. That the above is true was evident in several dogs in which certain branches caused inhibition and others acceleration; but the main trunk produced acceleration alone.

DISCUSSION. The present-day text-book conception that the glossopharyngeal nerve reflexly inhibits respiration has most likely been derived from Markwald's (7) description of the response obtained in the rabbit. In addition, Markwald correlated his findings with the observations of Meltzer (8), who came to the conclusion that respiration is inhibited during deglutition, on the basis of experiments performed upon himself. This correlation led Markwald to believe that deglutition inhibits respiration through the activity of the glossopharyngeal nerve. One of us has repeated Meltzer's experiments on himself, but could not confirm his findings. In a number of experiments carried out on dogs, the details of which shall be presented in a later publication, we could not confirm the generally accepted opinion that deglutition inhibits respiration through the glossopharyngeal nerve; for the effect of deglutition on respiration, which was a momentary inhibition, remained unaltered after both glossopharyngeal nerves were cut. On the basis of the observations reported above for the cat, it is also difficult to reconcile oneself to respiratory inhibition as the result of deglutition through the medium of the glossopharyngeal nerve. We did observe, however, that the glossopharyngeal nerve has an extremely marked inhibitory effect on the act of deglutition, as elicited by central stimulation of the superior laryngeal nerve.

SUMMARY

In the rabbit, stimulation of the central end of the glossopharyngeal nerve (with exception of the carotid sinus branch which may cause acceleration) gives rise to respiratory inhibition. In the cat the response is usually

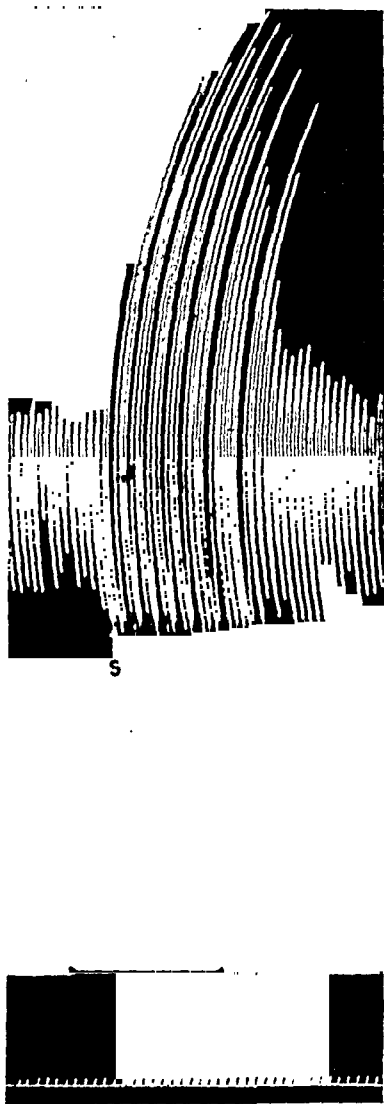


Fig. 5. Dog. Stimulation of the central end of the right glossopharyngeal nerve. Primary, 4.5 volts, secondary coil at 10 cm. Time, 1 second.

one of respiratory acceleration. More rarely inhibition, as in the case of the rabbit, is elicitable. In the dog the response is more variable, for a fair proportion of these animals respond with both inhibition and acceleration; while more respond with inhibition alone than with solely acceleration.

A preliminary series of experiments indicates that section of the glossopharyngeal nerves does not influence the effect produced by deglutition on respiration; but glossopharyngeal nerve section does markedly increase the number of swallowing movements elicited by stimulation of the central end of the superior laryngeal nerve.

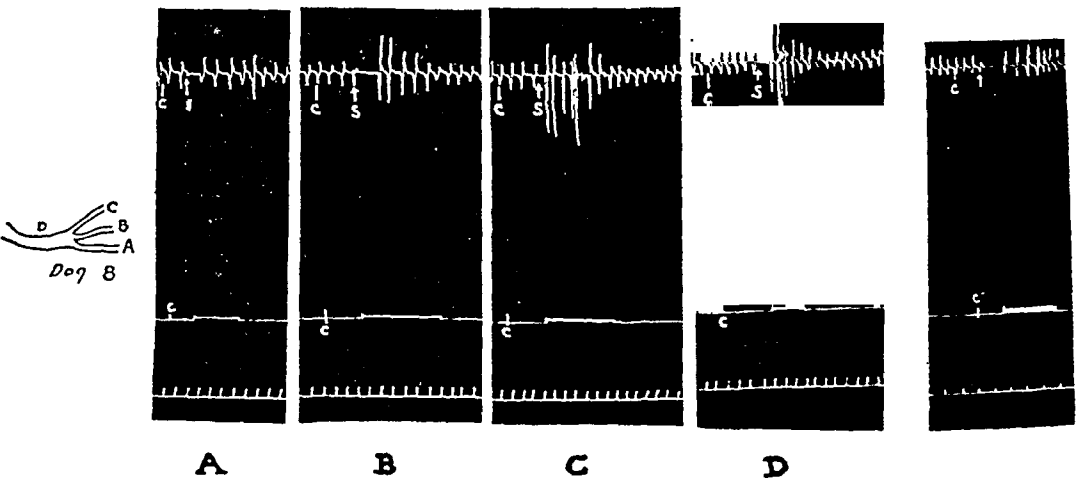


Fig. 6

Fig. 7

Fig. 6. Dog. A, B, and C, stimulation of the central end of branches A, B, and C of the left glossopharyngeal nerve. D, stimulation of the central end of the left glossopharyngeal nerve. Primary, 6 volts, secondary coil at 10 cm. Time, 1 second.

Fig. 7. Dog. Stimulation of the central end of the left glossopharyngeal nerve. Primary 5 volts. Secondary coil at 10 cm. Time, 1 second.

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THE COÖPERATIVE ACTION OF SYMPATHETIC NERVE IMPULSES, ADRENINE AND SYMPATHIN ON THE NICTITATING MEMBRANE OF THE CAT

A. C. LIU¹

From the Laboratories of Physiology in the Harvard Medical School

Received for publication May 24, 1935

The coöperation between sympathetic impulses and adrenaline, although long claimed to be possible (Elliott, 1912; Cannon, 1914), is supported by fragmentary experimental facts only. Besides the work of Elliott (*loc. cit.*) on blood pressure, some evidence is afforded by recent investigations on the nictitating membrane (n.m.) of the cat. Injected adrenaline was found to intensify the direct nervous effect (Rosenblueth and Rioch, 1933a), and secreted adrenaline increased the effect of sympathin—a humoral product of sympathetic nervous activity—from the lower abdominal chains (Rosenblueth and Cannon, 1932). Furthermore, sympathin from the heart reinforced that from the splanchnic area (Rosenblueth and Morison, 1934).

The studies mentioned, while suggestive of probable coöperation of the different sympathetic components in the organism, are not exhaustive. Moreover, no combinations of more than two sympathetic components have thus far been demonstrated. To supply such information, the present work was undertaken.

METHOD. Cats were used under dial ("Ciba," 0.8 cc. per kgm. intraperitoneally) or urethane (25 per cent, 6 cc. per kgm. intravenously). Isotonic contractions of the n.m.—either normal or denervated by resecting the corresponding superior cervical sympathetic ganglion two weeks previously—were recorded as described by Rosenblueth and Cannon (*loc. cit.*) with 2 to 4 grams of tension from a lever affording an 18-fold magnification. Shielded electrodes were used for stimulation. The duration of each stimulation was limited to a half minute. The stimulators were two Harvard induction coils and a neon tube unit. A possible mutual enhancing influence of the stimulators on each other when simultaneously applied was controlled.

Stimulation of the peripheral end of the cut cervical sympathetic supplied nerve impulses to the n.m. Adrenal secretion was elicited by direct

¹ Fellow of the Peiping Union Medical College, China.

excitation of a splanchnic nerve or reflexly from afferent activation of a sciatic nerve. Sympathin was obtained from the effectors supplied by the cardio-accelerator nerves, the hepatic nerves, and the lower abdominal sympathetic chains. Whenever a nerve to a certain organ or part was stimulated, its connections other than the one desired were sectioned if possible. The adrenals, when their secretion was not under investigation, were always removed.

Before the removal of the adrenals, cocaine (8 mgm. per kgm. intravenously) was injected to enhance the n.m. response. When reflex adrenal secretion was required, cocaine was omitted, since it abolishes the reflex. Curare was used, in addition to the anesthetic, to immobilize the animal whenever necessary. To avoid asphyxia, artificial respiration was employed.

RESULTS. 1. *Preliminary observations.* The n.m. response remained practically unchanged when a stimulus of a given intensity was repeatedly applied to the cervical sympathetic, but decreased progressively with chemical stimuli—adrenine or sympathin—under constant conditions (cf. Rosenblueth and Morison, *loc. cit.*). The rate of decrease of the chemical responses appeared to be related to the interval between stimulations and to the intensity of the stimulus. Thus the decrease in the responses was more marked with strong stimuli applied frequently. Only in rare cases was such a decrease not apparent.

The response to chemical stimulation differed from the nervous one in having a longer latency and a slower rate of contraction and relaxation. The following reasons may account for these differences. The nerve impulses are delivered to the n.m. directly, but the chemical agent does not become effective until an adequate concentration is reached in the blood stream. The localized nervous effects are readily dissipated, while the generalized chemical ones wear off but gradually.

The continuous rise of the n.m. and the delayed relaxation after strong nervous stimulation, reported by Rosenblueth (1932b), was also noted. The present observations, however, were made in cocainized animals. From the following data we believe that sympathin from effectors other than those in the n.m. was superimposed on the nervous effect in our case. Bacq (1933) showed that enough sympathin could be produced from a peripheral cervical sympathetic nerve to affect the retractor penis. We found that sympathin from the contralateral cervical sympathetic could occasionally cause a cocainized n.m. contraction. Sympathin from such a source, usually too little to act even on a cocainized n.m. by itself, readily called forth a bigger n.m. response when superimposed on a nervous effect (fig. 1).

From the above data, together with the fact that the n.m. responses increase asymptotically with respect to the strength of stimulation (Rosen-

blueth et al., 1932a, b, 1933b, 1934), it is clearly seen that, for such a study as this, submaximal stimulation should be used. First of all, submaximal stimulation will produce a practically pure nervous response which will be submaximal. Secondly, a submaximal response will allow further contraction of the n.m. in response to an added chemical influence. Finally, the chemical effect will not diminish so rapidly with submaximal stimulation.

2. *Summation of stimuli.* When stimulation of the cervical sympathetic just failed to evoke a response from the n.m., a superimposed chemical stimulus following the first one at varying time-intervals produced no effect. The stimulus therefore probably failed to set up any nerve impulses in the cervical sympathetic. When two subminimal chemical stimuli—sympathin from two sources or sympathin and adrenaline—were

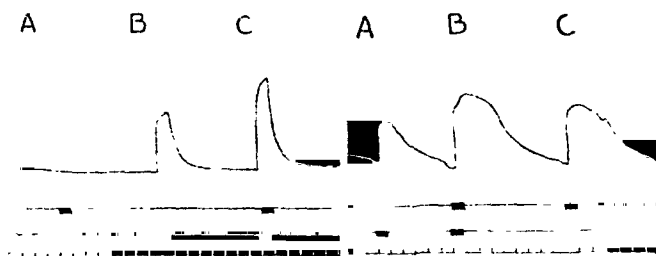


Fig. 1

Fig. 2

Fig. 1. Dial, cocaine and curare. Upper signal: stimulation of the contralateral cervical sympathetic. Lower signal: stimulation of the ipsilateral cervical sympathetic. In this and the succeeding figures the time signal records 30-second intervals.

Fig. 2. Previously denervated nictitating membrane. Dial and cocaine. Upper signal: stimulation of right cardio-accelerators. Lower signal: stimulation of left cardio-accelerators.

produced simultaneously, contraction, which might have been undetectable with either one alone, appeared. In the latter case, the inadequate concentration of one chemical substance must be made adequate by the presence of the other. The coöperation between a subminimal chemical stimulus and a minimal (or submaximal) chemical or nervous one was readily demonstrated (fig. 1).

When two stimuli—either both minimal or one minimal and one submaximal—were superimposed, a greater response resulted (figs. 2B, 3C, E and G). The height of the resultant contraction was greater than the sum of the two separated contractions. The duration was usually prolonged. The summated response increased with increasing intensities of the stimuli until finally the resultant response became different from the individual ones by a longer duration only. A second stimulus also short-

ened the latency and hastened the rate of contraction. The form of the resultant contraction curve with a predominant nervous component exhibited a sharper transition from contraction to relaxation than that with a predominant chemical one (compare E with A and D, fig. 3). The greater of the two chemical influences appeared to determine the shape of the resultant contraction curve (contrast G with D and F, fig. 3).

The above holds true with three stimuli when the combination of any two is considered as one stimulus and the third as the other (figs. 3B and 4A). In so far as the contractions of the n.m. are concerned, all the

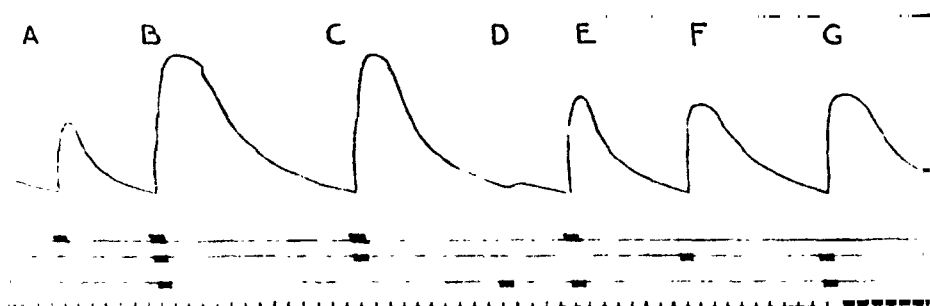


Fig. 3. Urethane and cocaine. Upper signal: ipsilateral cervical sympathetic. Middle signal: splanchnic supply to adrenal. Lower signal: hepatic nerves.

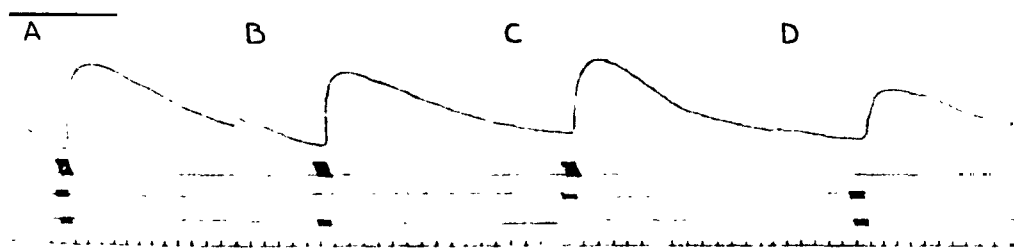


Fig. 4. Previously denervated nictitating membrane. Urethane and cocaine. Upper signal: splanchnic supply to the right adrenal gland. Middle signal: hepatic nerves. Lower signal: lower abdominal sympathetic chains.

effective stimuli have worked similarly. It is only on account of the different shapes of the responses that a distinction is made between the chemical and the nervous stimuli. The coöperation between different sympathetic components has been found demonstrable in any combination of two or three.

DISCUSSION. Our findings support the conclusion that sympathetic impulses, adrenaline and sympathin act synergetically (Cannon, 1934) on the n.m. and point to other interesting suggestions. The demonstration of subminimal chemical effects with the help of a minimal (or submaximal) chemical (or nervous) one (fig. 1) not only confirms the previous finding

of Rosenblueth and Cannon (*loc. cit.*) but also appears to be a delicate method for detection of small quantities of sympathin. If it can be proved to be true with sympathin I, which can be demonstrated in small quantities only, the method will be quite useful.

Since sympathin from stimulation of the contralateral cervical sympathetic sums in the cocaineized cat with the effects on the n.m. of the ipsilateral nerves (fig. 1), it has been pointed out above that a similar summation probably occurs also of the sympathin liberated locally at the n.m. with that liberated at other effectors, e.g., pilomotor or vessels, when only one cervical trunk is stimulated. A similar summation may occur at any other effector observed, when the nerve stimulated supplies other structures. This is a possible source of error which should be kept in mind when it is necessary to eliminate the action of any other factor than the nerve impulses evoking a given response.

The fact that simultaneous bilateral stimulation of the cardio-accelerators produces a bigger response of the n.m. than either one alone (fig. 2) indicates that the number of nerve fibers activated determines the amounts of sympathin produced.

SUMMARY

Summation of different sympathetic components—direct sympathetic nerve impulses, sympathin from other sources, and adrenaline—was studied by their simultaneous effects on the nictitating membrane of cocaineized cats. A cooperation of these diverse stimuli was found as indicated by effective summation of all combinations of two or three tried (figs. 1, 2, 3 and 4). A subminimal chemical stimulus, e.g., sympathin, is capable of increasing the response to any of the other excitatory agents (fig. 1). The method, therefore, affords an opportunity to reveal minute amounts of sympathin, otherwise undetectable.

I wish to express my gratitude to Prof. W. B. Cannon for his suggestion of the problem and to Drs. H. Davis and A. Rosenblueth for their counsel.

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PHYSICAL ACTIVITY AND THE BLOOD OF ALBINO RATS¹

EDWARD C. SCHNEIDER AND C. B. CRAMPTON

From the Department of Biology, Wesleyan University, Middletown, Connecticut

Received for publication May 21, 1935

In this study a comparison has been made of the blood of exercised and sedentary white rats in regard to the number of erythrocytes, content of hemoglobin, and number of reticulocytes per unit volume of blood. An attempt has also been made to discover whether or not in active animals the body might compensate for the increase of erythrocyte destruction by the production of red cells of greater resistance to the agents of blood destruction.

Animals ten weeks old were divided into two groups—the exercise and control groups. Each group was fed a well-balanced diet, supplemented by fresh lettuce two or three times a week to insure a vitamin sufficiency. The rats of the first group were placed in individual cylindrical revolving activity cages six evenings each week at about five o'clock and were released at eight the following morning. The food trays were removed from both groups during the period of exertion of the active group. The exercised rats ran on an average of about four miles per night; the females ran consistently somewhat longer than the males. Some of the exercised animals were started in December and others on February 4 and continued on the program until May 15. The average gain in weight during the period of investigation was 93.8 grams for the control animals, and 127.5 grams for the exercised animals. Hence it is evident that the exercised animals were at least as healthy as the sedentary animals of the control group.

Contrary to expectation, it was found that there was no difference in the bloods of rats subjected to an exercise period of several months' duration and the bloods of litter mates kept sedentary for an equal period of time. The final condition of the blood of each rat in erythrocytes, hemoglobin, and reticulocytes is given in table 1.

From this table it is seen that the number of erythrocytes and content of hemoglobin are practically the same for all animals regardless of the fact that one group had experienced between four and five months of considerable daily physical activity; while the other group, because of the smallness of their cages, had been restricted to little or no physical exercise. Appar-

¹ This research has been conducted with aid of a grant from the Charles Himrod Denison Fund.

ently the increased demand for oxygen by the exercised group was not enough to call for the circulation of an additional supply of red corpuscles or for corpuscles richer in hemoglobin. This latter statement seems true, since both the content of hemoglobin and the number of erythrocytes is practically the same for all animals, thus giving a color index approximately equal for both groups.

It was possible that the exercise taken by the active group might cause increased blood destruction to such an extent that the bone marrow would be compelled to increase its production of erythrocytes in order to maintain the normal supply of corpuscles; hence the reticulocyte count, which determines the number of young red cells, may be of interest. A study of table 1 shows no significant difference between the two groups of animals; therefore we may assume that the amount of exercise taken by our active group did not stimulate the bone marrow.

TABLE 1

CONTROL RATS				EXERCISED RATS			
Animal no.	Red cells	Hemo- globin in 100 grams blood	Reticulo- cytes for 10,000 cells	Animal	Red cells	Hemo- globin in 100 grams blood	Reticulo- cytes for 10,000 cells
	<i>millions</i>				<i>millions</i>		
1	8.47	14.27	5	9	8.15	13.8	8
2	7.45	13.03	16	10	7.35	13.18	6
3	7.70	13.12	9	11	8.27	13.51	12
4	8.46	15.24	14	12	8.80	14.23	14
5	8.68	13.42	9	13	8.00	13.77	11
6	8.62	13.14	19	14	8.56	13.71	17
7	8.38	13.9	14	15	8.44	13.35	11
8	8.92	13.56	5				
Average...	8.34	13.71	11.25		8.22	13.65	11.29

In view of Broun's (1923) observation that a marked erythrocyte destruction occurs during vigorous exercise in dogs previously kept sedentary, we had reason to expect that we should find evidence in our exercised animals of either increased resistance of the red blood cells to wear and tear or stimulated bone marrow activity.

If compensation took the form of the production of erythrocytes of greater resistance to the agent of blood destruction, then methods that test cell fragility should reveal a difference between our two groups of animals. Two methods were employed to test resistance: first, different dilutions of saponin by Eric Ponder's (1923) method and, second, different concentrations of hypotonic saline by the method of Simmel (1923). By both methods it was found that there was no significant difference in the resistance to hemolysis of the corpuscles of the two groups of animals.

The bone marrow of the two groups of animals was next inspected to discover any difference in the activity of the blood forming tissue. The femurs of the animals were excised. One bone of each rat was split lengthwise and the halves examined with a binocular dissecting microscope. The extremities of the bone from the opposite leg of each rat were cut off and an hypodermic needle inserted into the lumen of the shaft. Then through the shaft were forced two cubic centimeters of a solution of brilliant cresol blue dye dissolved in isotonic saline. This solution was a second time forced through the shaft, but in the opposite direction. In this perfusing fluid the number of cells and of reticulocytes was carefully counted. In both groups of animals an average of about 57 reticulocytes to 500 assorted bone marrow cells was found. This similarity was confirmed by inspection of the marrow lying within the split bones. Neither quantitative nor

TABLE 2
Blood changes during a compulsory run

ANIMAL NUMBER	RED BLOOD CELLS			HEMOGLOBIN		
	Rest	Exercise	Increase	Rest	Exercise	Increase
	millions	millions	per cent	per cent	per cent	per cent
16	8.11	8.87	9.4	115	121	5.2
17	8.14	9.51	16.8	117	129	10.3
18	8.85	10.51	18.8	113	129	14.1
19	8.14	9.50	16.7	115	129	12.2
20	9.86	10.32	4.7	120	129	7.5
21	9.04	9.35	3.4	116	128	10.3
22	8.85	9.53	7.7	120	134	11.7
23	8.89	9.26	4.2	120	127	5.8

developmental differences between the two groups of animals could be observed.

Since in every erythrocyte study made by us no difference was found in the bloods of rats subjected to an exercise period of several months' duration and the bloods of litter mates kept sedentary for an equal period of time, we are led to conclude that a moderate amount of physical activity does not stimulate the blood-forming centers of the rat. We estimate that during the first seven weeks of exercise the average distance run by each animal was about 160 miles. Before the end of the period of observation each animal had run about four times that distance. This amount of voluntary activity compares favorably with that reported by Slonaker (1925).

The effect of a single period of vigorous exercise. It is well known that in man, the horse, the dog, and other mammals a short period of strenuous physical effort results in a temporary concentration in the number of erythrocytes and in the hemoglobin of the blood. The white rat likewise

develops an increase in the number of circulating erythrocytes during a run. This was demonstrated on a group of normal rats by exercising them in a treadmill driven by an electric motor. The animals were compelled to run rapidly for from two to three and one-half minutes, long enough to cause them to show signs of fatigue and respiratory distress. Blood counts and hemoglobin determinations were made before the exercise while the animals were at rest, and directly after the muscular exercise. Previous to the experimentation, the rats were tamed in order to reduce excitement upon being handled. The data for before and after exercise are given in table 2. It is to be noted that an increase in the number of circulating erythrocytes and the content of hemoglobin is displayed in every instance following the muscular activity. The average number of erythrocytes for the animals while at rest was 8,740,000, and after exercise was 9,606,000, which is an average increase of 9.9 per cent. The content of hemoglobin rose from an average of 117 (Haldane scale) during rest to 129 after exercise, or an average rise of 10.2 per cent.

In view of the discussion regarding the explanation of how the increase in erythrocytes is brought about during a short period of exertion, the spleens were excised from a number of rats. The operation caused them to become anemic; hence they were fed liver and iron compounds in addition to the regular diet. While they improved markedly within a period of two weeks, they were not back to normal when experimentation had to end. Among them were three animals that had returned to within 8 and 10 per cent of their normal blood composition. These, as well as others, failed to develop the usual increase in the number of circulating red blood cells when compelled to run in the treadmill. It would seem, therefore, that the spleen in the normal rat serves as an important storehouse of reserve erythrocytes and that these may be brought into active circulation during a period of strenuous physical activity.

SUMMARY

Regular moderate physical activity over a period of months does not stimulate the hematopoietic tissue of the white rat to increased activity. This was demonstrated by the failure of the erythrocytes, hemoglobin, and reticulocytes to increase in a unit volume of blood. The resistance of the red corpuscles to hemolysis was also not modified by regular exercise.

A brief period of strenuous running produced a temporary rise in the erythrocyte and hemoglobin content of the blood of normal rats but failed to do so in splenectomized animals.

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THE CARBON DIOXIDE CONTENT AND COMBINING POWER AND pH OF CERVICAL LYMPH

J. WILLIAM HEIM AND OCTA C. LEIGH

From the Department of Physiology, Harvard School of Public Health, Boston, Mass.

Received for publication May 25, 1935

Information concerning lymph gases is meagre. The few observations that do exist are well summarized by Drinkler and Field (1933) and are principally concerned with the carbon dioxide content of thoracic duct lymph. Similar data for peripheral lymph are limited to the work of Tsuji (1934) who determined the carbon dioxide content of lymph in afferent and efferent vessels of the popliteal lymph node of rabbits. The data with respect to the hydrogen ion concentration of lymph are somewhat conflicting. von Krüger reports a pH value of 9 for thoracic duct lymph, while Gesell concludes that the blood is invariably more alkaline than the lymph. The only observations on peripheral lymph were made by Tsuji (1933) who reports values of 7.92 and 7.65 for lymph in the afferent and efferent vessels of the popliteal lymph nodes of rabbits. Details concerning the conditions under which the measurements were made are not given and corresponding values for simultaneously collected blood samples are not included for reference.

In view of the uncertain state of the literature with respect to the above mentioned properties of lymph, it was decided to investigate more thoroughly the carbon dioxide content and combining power and pH of cervical lymph of the dog. This lymph is easy to collect and can usually be obtained in sufficient quantity to provide duplicate or triplicate determinations for each observation.

TECHNIQUE. Sampling. Dogs weighing in excess of 20 kgm. were anesthetized with an intraperitoneal injection of nembutal. The cervical lymphatic trunks were exposed and cannulated. A small amount of heparin was placed in the tip of the cannulae to prevent coagulation. When analyzing for content, the lymph was collected anaerobically in specially constructed syringes (Behnke, Shaw, Shilling, Thomson and Messer, 1934) and approximately 1 cc. introduced directly into the chamber of the Van Slyke apparatus. Blood samples were withdrawn from the jugular vein by means of an oiled syringe and introduced under oil into the constricted centrifuge tubes of Myers and Muntwyler. Heparin was used as an anticoagulant. The blood was centrifuged and measured as described by Peters and Van Slyke (1932).

Methods. The carbon dioxide analyses of lymph and plasma were made by means of the Van Slyke manometric apparatus (Van Slyke and Neill, 1924) on 1 cc. samples. Duplicate or triplicate determinations were invariably made. Data for the dissociation curves were obtained by equilibrating the lymph and plasma in tonometer tubes with known tensions of carbon dioxide. The equilibration procedure was carried out for 20 minutes at 38°C. and the final composition of the gas mixture was determined by gas analysis. In the experiments in which the animal was permitted to breathe carbon dioxide, a gas mixture consisting of approximately 10 per cent carbon dioxide, 20 per cent oxygen, and 70 per cent nitrogen was

TABLE 1

The carbon dioxide combining power of separated plasma and cervical lymph

DOG NUMBER	SEPARATED PLASMA			CERVICAL LYMPH		
	CO ₂ tension, mm.Hg		$\Delta\text{CO}_{2_{60-30}}$	CO ₂ tension, mm.Hg		$\Delta\text{CO}_{2_{60-30}}$
	30	60		30	60	
	<i>volumes per cent</i>			<i>volumes per cent</i>		
1	53.6	59.8	6.2	59.1	62.8	3.7
2	50.4	55.3	4.9	51.6	56.6	5.0
3	56.5	62.8	6.3	57.3	61.9	4.6
4	56.2	62.3	6.1	59.4	63.7	4.3
5	56.5	61.8	5.3	61.8	65.3	3.5
6	58.1	63.5	5.4	57.5	61.8	4.3
7	59.2	65.0	5.8	60.5	66.2	5.7
8	50.8	56.0	5.2	52.5	57.3	4.8
9	54.0	59.0	5.0	57.2	61.5	4.3
10	62.3	68.4	6.1	66.8	71.5	4.7
11	51.2	56.1	4.9	54.1	59.5	5.4
12	61.6	67.5	5.9	63.6	68.4	4.8
13	63.5	68.2	4.7	66.2	70.3	4.1
14	53.4	57.4	4.0	56.6	60.5	3.9
17	54.0	59.5	5.5	60.1	64.5	4.4
Average..	56.1	61.5	5.4	58.9	63.4	4.5

administered through a tracheal cannula. Alveolar air samples were taken by means of the apparatus of Shaw and Messer (1930). The pH determination was conducted according to the method of Eisenman (1927) with the exception that the saturation technique of Austin was not used. The composition of the gas mixture in the tonometer tubes was determined by gas analysis. In order to apply this method to the determination of the lymph pH, the constants pK' and α of the Henderson-Hasselbalch equation must be known for lymph. Peters and Van Slyke (1931) give identical values of pK' for lymph and blood plasma. The solubility coefficient α of carbon dioxide in lymph is not known, but by inference from the work

of Van Slyke, Sendroy, Hastings and Neill (1928), we can safely assume that it does not differ greatly from that of plasma. These authors have shown that the deviation in the coefficient with respect to water and serum is principally due to the presence of three elements in the latter fluid: salts and protein which depress the solubility 3 and 2 per cent, respectively, and lipoids which increase the solubility about 4 per cent. The salt concentrations of plasma and lymph differ only slightly while the lymph has less than half as much protein and fat. From these relationships one can see that the solubility coefficient is approximately the same in lymph as in plasma. Furthermore, it can be shown that a variation of 5.7 per cent in the value of α causes an alteration in the pH of only 0.001, which is within

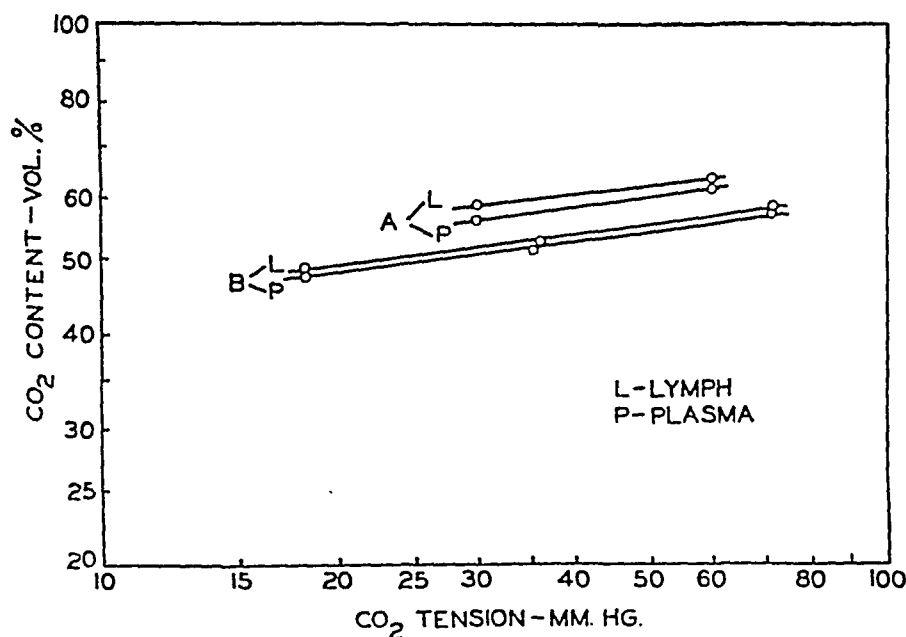


Fig. 1. Carbon dioxide dissociation curves of lymph and plasma. A, average of 15 experiments; B, typical experiment.

the limits of the experimental error. As we shall point out later, the saturation curve for lymph approximates a straight line when plotted on logarithmic coördinate paper. This means that both the lymph and plasma pH can be determined graphically on the same logarithmic pH chart. Lymph and plasma proteins were determined refractometrically.

DISCUSSION OF RESULTS. Figure 1 (B) shows typical dissociation curves for cervical lymph and separated plasma plotted on logarithmic coördinate paper. The values are based on the water contents of the fluids. It will be seen that the curves are approximately linear in shape, lie very close together, and have almost identical slopes. Table 1 gives values for the combining power of plasma and lymph of 15 animals at two points on the

dissociation curve. These figures were obtained by plotting the curves as in figure 1 (B) and determining the carbon dioxide contents at 30 and 60 mm. from the chart. These results are shown in figure 1 (A). The average difference in contents at these two tensions (ΔCO_{260-30}) is 5.4 volumes per cent for plasma and 4.5 volumes per cent for lymph. The value of

TABLE 2

The carbon dioxide content and tension and pH of cervical lymph and separated plasma

DOG NUMBER	CO ₂ CONTENT		CO ₂ TENSION		pH	
	Plasma	Lymph	Plasma	Lymph	Plasma	Lymph
	<i>volumes per cent</i>		<i>mm.Hg</i>			
6	57.0	57.3	41.0	40.0	7.39	7.41
7		60.7		43.0		7.40
8	49.1	51.4	38.0	33.0	7.36	7.44
9	52.8	56.8	40.3	38.0	7.37	7.42
10	63.3	67.8	56.0	45.0	7.30	7.43
11	50.0	53.7	43.6	41.0	7.31	7.37
12	62.6	63.3	54.0	41.4	7.31	7.44
13	62.8	65.2	51.9	38.6	7.32	7.48
14		56.3		38.0		7.42
17		56.0		45.4		7.34
Average..	56.8	58.8	46.4	40.3	7.34	7.41

TABLE 3

The carbon dioxide combining power of plasma and cervical lymph during carbon dioxide administration

DOG NUMBER	CO ₂ TENSION ALVEOLAR AIR	PLASMA		LYMPH	
		CO ₂ content	CO ₂ uptake per mm.Hg	CO ₂ content	CO ₂ uptake per mm.Hg
	<i>mm.Hg</i>	<i>volumes per cent</i>		<i>volumes per cent</i>	
15	38.8	57.5		59.9	
	85.9	67.0	0.202	67.9	0.170
16	36.8	62.3		65.3	
	91.1	73.5	0.206	73.1	0.144
18	41.2	58.4		60.0	
	90.4	68.5	0.205	69.6	0.195

$\Delta\text{CO}_{260-30} = 5.4$ of plasma is in good agreement with that of 5.6 which Eisenman found for human serum and is in exact agreement with the value of 5.4 which she found for cat's blood. We see from these charts that at a given tension of carbon dioxide, the lymph has a slightly higher combining power than plasma; while for a given change in tension, the lymph takes up slightly less carbon dioxide than the plasma.

Table 2 gives average values for the carbon dioxide content and tension and pH of separated plasma and cervical lymph of 10 animals. The carbon dioxide content of lymph varies from 51.4 to 67.8 volumes per cent with an average value of 58.8 volumes per cent. The carbon dioxide tension in the lymph ranges from 33.0 to 45.0 mm. of mercury and the average value is 40.3 mm. The average lymph pH was found to be 7.41 with a variation of 7.34 to 7.48. When compared with the corresponding pH value of 7.34 for separated plasma, it will be seen that the lymph is slightly more alkaline than the plasma.

Table 3 shows the results of three experiments in which the animal was permitted to breathe a gas mixture containing approximately 10 per cent carbon dioxide. It will be seen that the carbon dioxide uptake per millimeter of carbon dioxide tension is in every case slightly higher for plasma than for lymph.

SUMMARY

1. The carbon dioxide content and combining power and pH of cervical lymph of dogs have been determined.
2. The carbon dioxide dissociation curve of lymph was found to be a straight line when plotted on logarithmic coördinate paper.
3. For a given tension of carbon dioxide, the combining power of lymph is slightly greater than that of plasma.
4. The average difference between the carbon dioxide contents of lymph saturated at 30 and 60 mm. of carbon dioxide tension is 4.5 volumes per cent as compared with a corresponding value of 5.4 for separated plasma.
5. The average carbon dioxide content of the lymph of 10 dogs was found to be 58.8 volumes per cent.
6. The average pH of cervical lymph was found to be 7.41 with a corresponding value of 7.34 for separated plasma.

The authors are indebted to Dr. Cecil K. Drinker for advice and assistance throughout the course of this investigation.

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EFFECT OF POSTURE AND PROLONGED REST ON THE CARDIAC OUTPUT AND RELATED FUNCTIONS

SIDNEY A. GLADSTONE¹

*From the Medical Service of Dr. B. S. Oppenheimer, The Mount Sinai Hospital,
New York City*

Received for publication March 21, 1935

The cardiac output has been measured in these experiments by an application of the foreign-gas principle for the determination of the arterio-venous oxygen difference from which the cardiac output may be calculated, according to the equation:

$$\left. \begin{array}{l} \text{Cardiac output (liters of)} \\ \text{blood per minute) } \end{array} \right\} = \frac{\text{Rate of O}_2 \text{ consumption (cc. per min.)}}{\text{A-V difference (cc. of O}_2 \text{ per liter of blood)}}$$

The principles of the procedure and the method of calculation have been covered in detail by the writings of Krogh and Lindhard (1), (2) and Marshall and Grollman (3), (4).

The method is based on a comparison between the amount of oxygen and the amount of foreign gas absorbed by the mixed venous blood during its passage through the lungs. The validity of the results depends among other things upon the completion of the procedure before any appreciable amount of blood which has once been exposed to the foreign gas can complete a circuit and return to the lungs for a second time. The other technical difficulty is to obtain a homogeneous mixture in the gaseous system with which the blood leaving the lungs is assumed to be in equilibrium. This homogeneity of composition must be attained before the first sample is drawn, and with sufficient speed to allow time for comparing the amounts of oxygen and foreign gas absorbed before any appreciable recirculation occurs. The error due to recirculation has been estimated by Grollman as being quite small and negligible partly because of the compensating error due to quickening of the circulation by the deep breathing and the resultant slight increase of the oxygen content of the mixed venous blood. In 1932 Hamilton, Spradlin, and Saam (5) reported an enquiry into the basis of the acetylene method of determining the cardiac output, and by means of animal experiments, emphasized the error due to the

¹ Richard and Ella Hunt Sutro Fellow for Cardiovascular Research.

venous return of acetylene. The present writer after using the Marshall-Grollman procedure for one year (6) has felt it advisable to obtain more accurate information on this question in human subjects.

Two normal young men were used as subjects. The experiments were performed in the forenoon, one to four hours after breakfast. The subject sat in a chair for 10 minutes after which the rate of oxygen consumption was measured by means of a Sanborn-Benedict metabolism apparatus. The subject then rebreathed from a rubber bag containing an acetylene mixture, expiring to residual air before breathing from the bag, emptying the bag on each inspiration and breathing out as far as possible on each expiration. During the rebreathing several alveolar samples were drawn into evacuated tubes each at the end of a given expiration. The breathing and sampling were directed by an accurately timed phonograph record. In several experiments the inspired mixture was divided into two portions, the contents of the side bag being inspired first, the contents of the front bag following. This was done in order more quickly to attain a homogeneous mixture in the lung bag system (see below).

If and when any appreciable amount of acetylene-containing blood returns to the lungs for a second time, such recirculation will result in a depression in the rate of diffusion of acetylene from the lungs into the blood, and a concomitant rise in the calculated A-V difference. If the blood flow through the lungs during the rebreathing is fairly constant, the rate of diffusion of acetylene from the lungs into the blood should always be proportional to the concentration of the gas in the lungs. Neglecting slight changes in total volume of the gaseous system, the rate of decrease of the acetylene concentration should follow the equation,

$$-\frac{dC}{dt} = KC$$

where C is the concentration of the acetylene in the lung bag system at the time t , and K is the diffusion constant. For any interval of time t (between t_1 and t_2) one obtains

$$\frac{\log C_1 - \log C_2}{t} = 0.4343K$$

Accordingly, if the rate of diffusion of acetylene is proportional to the concentration, the latter should follow an exponential or parabolic curve against time as the abscissa, and the logarithms of the acetylene concentrations should follow a rectilinear curve.

The results obtained in ten experiments on two subjects demonstrate a rise in the calculated A-V difference as the breathing continues, and a fall in the rate of diffusion of C_2H_2 from the lungs into the blood. A typical

experiment is summarized in table 1. The logarithms of the C_2H_2 concentrations charted against time as the abscissa, in all experiments indicate a decrease in the slope of the curve occurring within ten to twelve seconds after the rebreathing has begun. Since the slope of the curve indicates the rate of diffusion of the gas, the change in slope of the curve indicates the time at which recirculation begins in appreciable amounts. Since the normal complete circulation time is about 22 seconds (7) the present findings indicate that the deep rapid breathing has approximately doubled the rate of blood flow through the lungs.² This conclusion is confirmed by calculation of the volume of oxygen or of acetylene removed from the lung-bag system by the blood during the first ten seconds, before recirculation

TABLE 1
Results of typical rebreathing experiment

	BREATH NUMBER					
	2	4	6	8	10	12
CO ₂	4.69	5.89	6.54	6.86	7.17	7.44
C ₂ H ₂	12.53	10.87	9.58	8.73	7.90	7.20
O ₂	19.77	18.85	17.91	17.21	16.32	15.33
N ₂	63.01	64.39	65.97	67.20	68.61	70.03
Time interval (second).....	5-10		10-15	15-20	20-25	25-30
A-V difference.....	56.5		63.4	63.8	71.1	80.6
K (diffusion constant).....	0.02842		0.02526	0.01859	0.01998	0.01856
Rate of C ₂ H ₂ diffusion.....	125.1		111.2	80.13	84.38	76.56

Bag contents before rebreathing:

	cc.
O ₂	270
C ₂ H ₂	600
Air.....	1530
Total.....	2400

has begun. To account for the volumes of each gas removed, the mixed venous blood must pass through the lungs at a rate of 10 to 12 liters per minute. The promptness (10-12 sec.) with which recirculation begins necessitates a very rapid attainment of uniformity in the gaseous system to permit the drawing of the first sample within 3 or 4 seconds, leaving a period of five or six seconds during which the gaseous diffusion from the lungs into the blood may be measured.

Two methods of achieving this result have occurred to the writer. One is to wash out the dead space into a side tube after the first deep inspira-

² Gladstone, S. A. and Dack, S. Proc. Soc. Exp. Biol. Med. 32: 1315, 1935. Gladstone, S. A., *ibid.*, p. 1319.

tion, using about 800 to 1000 cc. to accomplish this (8), then to turn the valve so that the remainder of the first deep expiration enters the breathing bag and consists entirely of alveolar air. The other method to *approximate* uniformity is to start the rebreathing after having expired to residual air, by inhaling in succession the contents of two separate bags, the composition of which has been adjusted so that the mixture in the second bag (the last portion of which fills the dead space) approximates in gaseous composition the expected alveolar air resulting from a mixture of the contents of the first bag and the residual lung air. In the technique to be described both these methods have been incorporated, the advantage being that the error due to possible incomplete washing of the dead space will be reduced to a minimum because the gaseous composition of the dead space has been previously made to approximate that of the alveolar air.

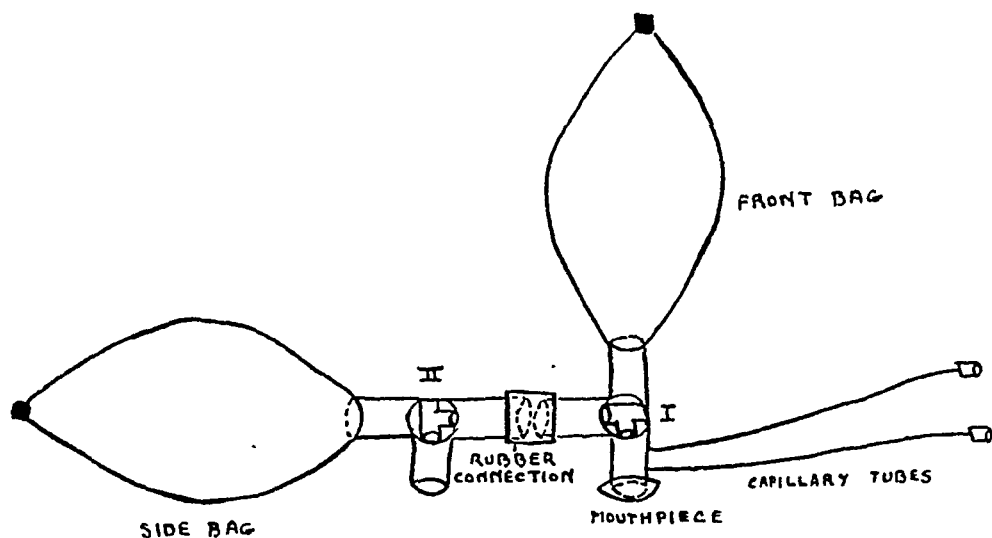


Fig. 1

METHOD. The apparatus consists of two three-way aluminum valves (bore 2 cm.), two rubber breathing bags (capacity 4 liters) connected as shown in the diagram (fig. 1) and supported on iron stands so that the subject with his lips over the mouth piece will have one bag directly in front, and the other bag to his left. Brass or lead tubes 15 cm. in length and of capillary (0.5 mm.) bore permit the drawing of samples from the mouth side of the valve into evacuated sampling tubes. After the valves have been turned to close both bags, the gases are introduced as follows:

Front bag cc.		Side bag cc.
825	Air	1300
55	CO ₂	50
120	C ₂ H ₂	500
	O ₂	150
Total . . . 1000		Total . . . 2000

A vital-capacity spirometer may be used for measuring the volume of air introduced, and a 250 cc. glass graduated syringe for measuring the other gases. If commercial acetylene is used, it must be washed to remove the traces of acetone and other impurities as recommended by Grollman (4). In preparing the mixtures it is preferable first to introduce the required amount of air into each bag to avoid at any time a pure oxygen-acetylene mixture. In normal subjects with a vital capacity of 3500–4000 cc. the volumes indicated are satisfactory. If the vital capacity rises much above 4000 cc., the volume of gas in the front bag may be increased, the percentages being kept the same.

The rebreathing. The subject with his lips over the mouthpiece breathes quietly through his nose until the spring nose-clip is applied and the subject exhales through the mouth to residual air, the air leaving through the side arm of valve II (fig. 1). After the subject has expired to residual air, valve II is turned so that the subject inspires the contents of the side bag. Immediately the side bag is empty, valve I is turned so that the contents of the front bag may be inhaled. When the front bag has been emptied, valve I is again turned to permit approximately the first liter of the ensuing expiration to be discarded into the side bag, the remainder of the expired air being then directed by another turn of valve I into the front bag. During this procedure the subject has taken a deep inspiration, emptying both bags, and a deep expiration, the first portion of which has been discarded. The operator has turned valve II once, and valve I three times. This preliminary period can be finished in 3.5 to 4.0 seconds after a little practice on the part of the operator and without undue haste on the part of the subject, whose only task after expiring to residual air is to inhale deeply and then exhale deeply. At the end of the latter expiration the first alveolar sample is drawn. The subject then takes two complete deep breaths out of and into the bag, emptying the bag on each of the two inspirations, and then breathing out as far as possible. These two complete respiratory cycles should take about 5 seconds. At the end of the second deep expiration, the second alveolar sample is drawn. The total duration of the test, i.e., the interval between the first inspiration of the foreign-gas mixture and the drawing of the second gas sample is $8\frac{1}{2}$ to 9 seconds. Errors due to recirculation of blood containing the foreign gas will become appreciable at ten seconds or shortly thereafter.

Mixing. Before applying the method described it is well to test the adequacy of the technique for attaining uniformity of gaseous composition prior to drawing the first sample. If the gaseous system is of heterogeneous composition immediately before the drawing of the first sample, such heterogeneity may be demonstrated by simultaneously drawing the first sample from both the proximal and distal ends of the rebreathing bag, and calculating the A-V difference from either of these as the first sample

paired with the usual second sample drawn after the last breath. If homogeneity of gaseous composition has not been attained the A-V differences thus calculated should show wide divergence. If homogeneity has been attained, the two samples drawn from opposite ends of the bag should show only a slight difference because the mouth sample has left the lungs a fraction of a second later than the distal sample, and since gaseous diffusion has continued during that time, one will find a slightly higher CO_2 and lower O_2 and C_2H_2 concentration in the mouth sample. These changes, resulting from diffusion across the alveolar epithelium should not affect the calculated A-V difference.

Experimental. Seven determinations of the A-V difference were made on three subjects, reclining in a deck chair, one to two hours after break-

TABLE 2

Effect of drawing first alveolar sample from opposite ends of rebreathing bag

SUBJECT	PULSE RATE PER MINUTE	BLOOD PRESSURE	OXYGEN CONSUMP- TION	ARTERIO-VENOUS OXYGEN DIFFERENCE OF BLOOD		DIFFERENCE BETWEEN A AND B	CARDIAC OUTPUT	
				A	B		I	II
		<i>mm. Hg</i>	<i>cc. per minute</i>	<i>cc. per liter</i>	<i>cc. per liter</i>		<i>liters per minute</i>	<i>liters per minute</i>
A. L.	64	108/65	256	49.7	53.9	4.2	5.2	4.8
A. L.	76	106/75	284	50.4	49.7	0.7	5.6	5.7
A. L.	68	112/72	258	51.3	52.3	1.0	5.0	4.9
S. D.	76	115/70	259	43.8	50.2	6.4	5.9	5.2
S. D.	70	105/72	247	43.6	46.0	2.4	5.7	5.4
S. A. G.	88	116/80	252	43.1	45.8	2.7	5.9	5.5
S. A. G.	88	120/76	270	50.3	50.1	0.2	5.4	5.4
Average.....						2.5	5.5	5.3

fast. The first sample was simultaneously drawn from opposite ends of the rebreathing bag. In column A (table 2) are given the results of the conventional samples. In column B, the additional first distal sample has been paired with the second sample. The average difference of about 5 per cent in the two sets of determinations may be attributed to the combined errors of inadequate mixing and gas analysis, and since the latter alone may easily account for the differences observed, one may assume that the error due to inadequate mixing in the method described is either negligible or non-existent.

RESULTS. The method as described has been applied in 34 consecutive determinations to study the effect of posture and prolonged rest on the cardiac output and related functions in six normal young men, fasting and at rest. Each subject was studied while sitting upright in a straight-back

chair, and usually on the following day while reclining in a deck chair. Since the findings are similar in all subjects, a detailed protocol is presented for the first subject (table 3) and the findings for the group are summarized in tables 4 and 5. The results indicate a difference in all circulatory functions listed (not the rate of O_2 consumption) when the two positions studied are compared. In the sitting position the pulse rate, systolic pressure, diastolic pressure, and A-V difference are distinctly higher, while the

TABLE 3

Subject, A. L. Male, aged 33 years. Height, 166 cm.; wt. 57 kgm.

TIME	PULSE RATE PER MINUTE	BLOOD PRESSURE	OXYGEN CONSUMP- TION	ARTERIO- VENOUS OXYGEN DIFFERENCE	CARDIAC OUTPUT	REMARKS
<i>a.m.</i>		<i>mm. Hg</i>	<i>cc. per minute</i>	<i>cc. per liter</i>	<i>liters per minute</i>	
8:50						2/6/35 (seated in chair)
9:25	62	112/85				
9:45	63	110/82				
9:56			215			
10:00	61	110/85				
10:07				65.4	3.3	
10:37			222			
10:40	61	110/85				
10:45				68.3	3.3	
11:20			222			
11:30	57	110/85				2/7/35 (reclining in deck chair)
11:31				65.4	3.4	
9:00						
9:50	58	100/68				
10:05			194			
10:10	60	102/65				
10:15				41.2	4.7	
10:47			206			
10:55	57	105/80				
11:00				58.0	3.6	
11:34			217			
11:40	55	105/75				
11:55				58.7	3.7	

cardiac output, systolic output, and pulse pressure are distinctly lower when compared with the results found in the recumbent position. As rest is prolonged in either position, the pulse rate and cardiac output progressively decrease. It is significant that the changes in systolic output whether produced by postural difference or by prolonged rest are accompanied by similar changes in the pulse pressure (9).

DISCUSSION. The present method for determining the arterio-venous

oxygen difference and cardiac output does not differ in principle from the nitrous oxide method of Krogh and Lindhard (2), but follows more closely the rebreathing procedure of Marshall and Grollman. The essential feature of the present method is its termination within 8.5 to 9 seconds to avoid the error due to recirculation which begins within 10 seconds or promptly thereafter, as demonstrated by the rebreathing experiments here reported. To complete the procedure within 10 seconds a rapid

TABLE 4

Effect of posture and prolonged rest on cardiac output (liters per minute)

SUBJECT	SITTING			RECUMBENT		
	I hr.	II hr.	III hr.	I hr.	II hr.	III hr.
A. L.	3.3	3.3	3.4	4.7	3.6	3.7
S. D.	4.9	3.4	3.2	4.2	4.1	4.6
S. A. G.	4.3	3.4		5.3	4.4	
A. P.	4.7	4.7	3.9	4.1	4.8	3.8
S. Z. S.	3.3	3.7	2.9	4.4	4.0	4.5
J. B.	3.5	3.3	3.1	3.8	4.1	3.7
Average.	4.0	3.6	3.3	4.4	4.2	4.1

TABLE 5

Averages of circulatory functions in six subjects as affected by posture and prolonged rest

FUNCTION	SITTING			RECUMBENT		
	I hr.	II hr.	III hr.	I hr.	II hr.	III hr.
Pulse rate per minute.	66	65	63	60	59	58
Systolic blood pressure.	114	113	113	104	107	107
Diastolic blood pressure.	88	88	90	71	75	75
Oxygen consumption, cc. per minute.	217	214	216	210	219	218
A-V difference.	56	60	66	49	53.6	54.4
Cardiac output, liters per minute.	4.0	3.6	3.3	4.4	4.2	4.1
Systolic output, cc.	61.3	59.5	51.0	71.8	70.3	70.6
Pulse pressure.	26	25	23	33	32	32

method for attaining homogeneity of gaseous composition within 3.5 to 4.0 seconds has been imperative. Such a method has been described and its adequacy tested. The writer believes that after removal of the error due to recirculation, the foreign-gas method is rendered more accurate as to absolute values obtained, and more sensitive in picking up small changes in arterio-venous oxygen difference. This belief is strengthened by a comparison of the results here reported with those obtained by Grollman

(10) who found no change in the cardiac output in different postures, although practically all other workers using different methods have reported results which agree with the present observations (11).

The finding of a higher cardiac output in the recumbent position, and a decreasing cardiac output as rest is prolonged in either the sitting or recumbent position cannot be attributed to any metabolic differences because no consistent differences in metabolic rate were observed. The observed changes probably result from the better venous return to the heart in the recumbent position, and the decreased venous return as muscular relaxation is increased by prolonged rest.

The effect of posture on the cardiac output (and related values) and especially the effect of prolonged rest indicate the importance of accurately defining these factors in the attempt to establish normal values or in the comparative study of abnormal conditions produced by experiment or by disease.

SUMMARY

A method based on the foreign gas principle for determination of the arteriovenous oxygen difference (and cardiac output) has been described. Its distinguishing feature is its termination within ten seconds in order to avoid the error due to recirculation of appreciable amounts of blood, which has been demonstrated to occur at that time or promptly thereafter.

The method as applied in 34 consecutive determinations in six normal young men has demonstrated a cardiac output 10 to 25 per cent higher in the recumbent as compared to the sitting posture. In either position, although basal conditions may be attained in one hour as indicated by the constancy of the rate of oxygen consumption, the cardiac output progressively decreases as rest is prolonged for a second and third hour. These changes run parallel with the observed changes in pulse rate and pulse pressure, the latter following closely the systolic output.

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THE EFFECT OF LACTOGENIC HORMONE PREPARATIONS ON THE BLOOD SUGAR LEVEL OF RABBITS AND MONKEYS

W. O. NELSON, C. W. TURNER¹ AND M. D. OVERHOLSER

From the Departments of Anatomy and Dairy Husbandry, The University of Missouri

Received for publication May 20, 1935

The observations of Houssay and Biasotti (1931), Barnes and Reagan (1933), Baumann and Marine (1932) and others that some anterior pituitary factor is involved in carbohydrate metabolism have added an additional rôle to the array of functions attributed to the anterior lobe. The possibility that the blood sugar-raising factor of the anterior lobe might be identified with some recognized principle has been considered, but very little evidence has appeared concerning such an alliance. The extracts used by the above workers were rather crude preparations and undoubtedly represented a complex of pituitary factors. The partially purified growth hormone extracts used by Evans, Meyer, Simpson, and Reichert (1933) would appear to fall in the same category. Lucke, Heydemann, and Duensing (1933) report that their thyrotropic extract does not induce glucosuria. Ferrill, Rogoff, Barnes and Scott (1934), as well as others, have presented evidence which suggests that the hypophyseal relation to carbohydrate metabolism may be exerted by way of the adrenals. However, insofar as we are aware, no reports have appeared concerning the effects of a purified adrenotropic extract upon carbohydrate metabolism.

We have been attracted by the possibility that the blood sugar-raising factor might be identical with the lactogenic hormone since the secretion of milk demands the utilization of quantities of sugar. Support was accorded this possibility by the observation that certain female dogs injected with a crude pituitary extract which induced glycosuria also lactated.

We have sought to investigate this question on monkeys and rabbits by means of various anterior pituitary extracts of proven lactogenic potency. The Schaeffer-Somogyi (1933) method of sugar determination was used in the estimation of blood and urine sugars. The monkey urines were twenty-four hour samples while the rabbit urines were obtained by catheterization.

¹ From the Dairy Husbandry Department, Agricultural Experiment Station, University of Missouri, Journal Series Paper no. 404.

The monkeys used were all sub-adult female macaques. Two were otherwise untreated and two had been totally depancreatized. The diabetes of the latter two animals was controlled by the daily injection of oestrin (Nelson and Overholser, 1934). Two extracts of known lactogenic potency were tested on these animals. Extract B contained one rabbit unit² of lactogenic hormone in 3 cc., while extract A³ had a potency of one rabbit unit per 15 mgm. As made up in solution the potency of the latter was one unit in $1\frac{1}{2}$ cc. Although we have not studied these extracts for their content of all of the supposed pituitary hormones we have found no significant gonadotropic (follicle-stimulating) or thyrotropic effects. Riddle and his co-workers (1933), who have examined the effects of prolactin extracts prepared by a similar method, state that there is little or no contamination of such preparations by the other recognized pituitary principles. The extracts used in the present study when injected in the monkeys in amounts of 3 to 6 cc. daily over periods of 4 to 7 days induced no significant changes in the blood sugar level and no evidence of glycosuria. Lactation did not occur in these animals despite the potency of the extracts. Since the mammary glands of these monkeys were not developed sufficiently to support lactation this finding was not surprising. Each of these animals later showed hyperglycemia and glycosuria following the administration of a crude alkaline extract of sheep pituitaries or an acetic acid extract of beef hypophyses (Nelson and Overholser, 1934). The sheep pituitary extract contained appreciable amounts of lactogenic hormone, but the beef gland extract showed no activity when tested on the pseudo-pregnant rabbit.

In studying the effects of extracts A and B upon the rabbit we have used pseudopregnant and oestrous animals. The animals were made pseudopregnant by the intravenous injection of Antuitrin-S.³ This treatment, as is well known, induces ovulation and corpus luteum formation. The mammary glands of such animals undergo marked proliferation and are completely responsive to the lactogenic hormone. The oestrous animals represent isolated adult females receiving no preparatory treatment. The value of using pseudopregnant animals in the present study is apparent since we thus were able to check the lactogenic potency of the extracts while we were determining their capacity to elevate the level of the blood sugar. The oestrous animals, while not as suitable for the study of the

² The rabbit unit is defined by Gardner and Turner (1933) as the minimum amount of extract which injected during a period of seven days at daily intervals is required to induce a plus three or plus four lactation in pseudopregnant rabbits. We have used five rabbits on a given level of hormone and demanded the above response in at least three of the five animals.

³ This material was supplied to us through the kindness of Dr. Oliver Kamm of Parke, Davis & Co.

lactogenic hormone, might be expected to respond to any blood sugar-elevating activity present in the extracts.

The usual procedure employed on the rabbits was the injection of a given amount of hormone over a period of 6 to 10 days. The mammary glands were examined at the end of the experiment and the lactation rated according to the method of Gardner and Turner (1933). At least one and often two blood sugar determinations were made on each animal prior to the initiation of treatment. Thereafter, several determinations were made on each animal during the test period.

Seven pseudopregnant and three oestrous animals were tested on extract A. The details of treatment and blood sugar findings for these animals are shown in table 1. It will be noted that with the exception of

TABLE 1
Data on rabbits injected with extract A

RABBIT NUMBER	DAILY DOSE	BLOOD SUGAR VALUES (MG. PER CENT)								
		Control	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Days 7-10	Lactation rating
Pseudopreg- nant										
1	$\frac{1}{2}$ Rb. U.	88; 75	95	90	85	73		95		Plus 4
2	1 Rb. U.	105; 90	90		115	75	88		110; 95	Plus 4
3	1 Rb. U.	82	85	95	80	72		88		Plus 4
4	2 Rb. U.	75; 70	98	115	110	78	85	105		Plus 4
5	2 Rb. U.	72	80	65	75		70	85	78; 80	Plus 3
6	3 Rb. U.	80; 92	72	72	85	80	88	75		Plus 4
7	3 Rb. U.	90	78	85	75	70		88	105; 88	Plus 4
Oestrous										
8	1 Rb. U.	80	95	78	88	110	85	88		Slight
9	2 Rb. U.	75	82	68	72	95	85	88	92; 82	Plus 1
10	3 Rb. U.	95; 82	80	88	95	100	72	80		None

rabbit 4 there is no evidence that the extract had any tendency to elevate significantly the level of the blood sugar. Although rabbit 4 showed a rather consistently increased blood sugar we are inclined to consider this single instance as of doubtful significance. As a whole the individual readings are of the order that one might expect to find in a similar group of untreated rabbits. In several of these rabbits a number of urine samples were obtained by catheterization. None of these urines showed evidence of sugar.

As was to be expected the pseudopregnant rabbits all showed excellent lactation. The oestrous animals showed a variable degree of lactation. Rabbit 9 had a fair plus one (ducts filled with milk) lactation, while rabbit 8 showed only a scanty secretion and rabbit 10 did not lactate. Further-

more, the lactogenic hormone did not induce any detectable increase in mammary gland proliferation.

Extract B was tested on six pseudopregnant and three oestrous animals with results similar to those found for extract A. In no instance did the blood sugar readings vary significantly from the normal range. The degree of lactation observed in these animals was of the same order as has been noted for the preceding group.

SUMMARY

In the present study preparations of the lactogenic hormone which were believed to be relatively free of other recognized pituitary principles failed to induce significant alterations in the blood sugar level of monkeys and rabbits.

Four monkeys showed no elevation of the blood sugar level and no glycosuria during the test periods. Thirteen pseudopregnant and six oestrous rabbits were treated for periods up to 10 days. No significant alteration in the blood sugar level occurred and no sugar was found in the urines examined.

It would appear from these studies that the lactogenic hormone is not allied with the blood sugar-elevating factor of the anterior pituitary. Previous studies have shown that lactogenic hormone preparations may be relatively free of the growth, thyrotropic, adrenotropic, and follicle-stimulating factors.

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